The Role of Autologous Fat Grafting For Breast Reconstruction

by

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ABSTRACT

The past decade has seen increasing interest in the development and use of autologous fat grafting for breast reconstruction. This thesis presents a review of the literature on the broad application and science of fat grafting and a review of the clinical literature on the cohort of women who have undergone mastectomy for breast cancer and subsequent fat grafting for breast reconstruction. The areas explored in the systematic review include efficacy, fat grafting and breast cancer oncogenesis, complications and outcomes.

Whether there is a need for the option of autologous fat grafting in women who have undergone breast conserving surgery was investigated (Chapter 3). Breast conserving surgery is the current mainstay of treatment for early breast cancer and outcomes were studied using the BREAST-Q patient reported outcome measure. It was identified that up to 15% of women with breast conserving surgery would consider reconstructive surgery and an appropriate procedure for minor defect correction is autologous fat grafting. Interestingly when controls, women with breast conservation, women with mastectomy alone and women with mastectomy and reconstruction were compared, women with mastectomy and reconstruction reported outcomes at least as good as those with breast conserving surgery.

A study of the efficacy of the BRAVA® external expander device and autologous fat grafting for breast reconstruction in women who have undergone breast conserving surgery and total mastectomy is described in Chapter 5. The idea for this project came from a conference presentation on the improved efficacy and enhanced fat graft 'take' when the BRAVA® external expander is used in combination with fat grafting. This study used

previously validated quantitative measures of outcome including, magnetic resonance imaging, 3D laser scanning and the BREAST-Q to quantify the proportion of fat graft retained in reconstructive cases and measure outcome as perceived by the patients. Rates of fat graft retention were found to be poorer than those described by the original group using the device with the mean proportion of fat retained being 48% at 12 months post-injection.

Since the start of the modern era of autologous fat grafting (in the early 2000s) there has been ongoing debate in the plastic surgery and breast cancer community regarding laboratory and clinical evidence for risk of oncogenesis and the interaction of adipose derived stem cells in the breast cancer microenvironment. This debate was the stimulus for the laboratory study (Chapter 7) investigating the morphology and behaviour of both benign and malignant breast cells in the presence of autologous fat from various sources. Although the laboratory studies were preliminary in nature the growth rates of benign breast cells in 2D culture were found to be higher in media containing autologous fat compared to those in baseline media and there were some differences in cell morphology between those in control medium and those in media containing autologous fat.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree of diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed:

Dr Benjamin Henry Lamus Howes MBBS

Dated at on

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DEDICATION

In loving memory of my brother Alejandro Parraga (05.10.1971- 23.02.2007). Who served as my role model by demonstrating focus, determination and academic achievement during his own studies. Then with his strength of character during the course of his illness, taught me the meaning of courage.

PUBLICATIONS

The following is the list of publications which were generated from the work contained within this thesis.

1. HOWES, B. H., FOSH, B., WATSON, D. I., YIP, J. M., EATON, M., SMALLMAN, A. & DEAN, N. R. 2014, Autologous Fat Grafting for Whole Breast Reconstruction: A Case Report
   Abstract publication: ANZ Journal of Surgery Volume 84 Supplement 1 (PR121P) pg 165.

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3. HOWES, B. H., FOSH, B., WATSON, D. I., YIP, J. M., EATON, M., SMALLMAN, A. & DEAN, N. R. 2014. Autologous fat grafting for whole breast reconstruction. *Plast Reconstr Surg Glob Open*, 2, e124.

4. HOWES, B. H., WATSON, D. I., XU, C., FOSH, B., CANEPA, M. & DEAN, N. R. 2016. Quality of life following total mastectomy with and without reconstruction versus breast-conserving surgery for breast cancer: A case-controlled cohort study. *J Plast Reconstr Aesthet Surg,* 2016 Sep;69(9):1184-91. doi: 10.1016/j.bjps.2016.06.004. Epub 2016 Jun 18

5. HOWES, B.H.L, WATSON, D.I., FOSH B, YIP JM, KLEINIG P, DEAN NR. Magnetic resonance imaging versus 3-dimensional laser scanning for breast volume assessment after breast reconstruction. Ann Plast Surg. 2017 Apr;78(4):455-459. doi: 10.1097/SAP.0000000000000890

PRESENTATIONS

The following is the list of presentations which were generated from the work contained within this thesis.

1. Whole Breast Reconstruction with Autologous Fat Transfer: A Case Report.
   Presenter: Benjamin Howes
   Verbal presentation at the Annual Scientific Meeting in Barossa Valley - August 17th 2013 (Local Meeting)

2. Autologous Fat Grafting for Whole Breast Reconstruction
   Presenter: Benjamin Howes
   Poster Presentation: Breast Co-ordinated Care Conference Washington D.C. 2014 (International Meeting)

3. Autologous Fat Grafting for Whole Breast Reconstruction: A Case Report
   Presenter: Benjamin Howes
   Verbal presentation RACS Annual Scientific Congress and ANZCA Annual Scientific Meeting, Singapore May 5th -9th 2014 (International Meeting)

4. *Well-being and Satisfaction with Breasts in Women who have Undergone Partial Mastectomy*
   Presenter: Benjamin Howes
   Poster Presentation: Breast Co-ordinated Care Conference Washington D.C. 2014 (International Meeting)

5. *Well-being and Satisfaction with Breasts in Women who have Undergone Partial Mastectomy*
   Presenter: Benjamin Howes
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6. ***Efficacy of BRAVA Device and Autologous Fat Grafting for Breast Reconstruction in Partial & Total Mastectomy Patients.***
   Presenter: Benjamin Howes
   Verbal Presentation Plastic Surgery Congress 6-10 May 2015 Brisbane Convention Centre- Hosted by Australia Society of Plastic Surgeons.

7. ***Quality of Life following mastectomy versus partial mastectomy for Breast Cancer***
   Presenter: Benjamin Howes
   Verbal Presentation Plastic Surgery Congress 6-10 May 2015 Brisbane Convention Centre- Hosted by Australia Society of Plastic Surgeons.

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Abbreviations

AFT- Autologous fat transfer

ADSC- Adipose derived stem cells

MSC- Mesenchymal stem cells

MMG- Mammogram

US- Ultrasound

MRI- Magnetic Resonance Imaging

CAL- cell assisted lipotransfer

SVF- Stromal vascular fraction

LVI- Lymphovascular invasion

LD- Latissimus Dorsi

TRAM- Transverse rectus abdominis muscle

TC- Tissue Culture

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Chapter 1. Literature review

1.1 Background

In Australia the incidence of breast cancer has increased by approximately 1-2 per cent per annum in the past decade.(Early and Breast Cancer Working Group, 2001, A, 2009) The Australian Institute of Health & Welfare (AIHW) reported 5,317 cases in 1982 rising to 13,688 in 2009. This figure makes breast cancer the most common type of cancer affecting Australian women. The incidence is expected rise to 17,210 by 2020 (Australian Institue of Health and Welfare, 2012). Approximately 25 per cent of women diagnosed are under the age of 50, 50 per cent between 50-69 years of age. The overall risk of developing breast cancer before the age of 85 is one in eight (Australian Institue of Health and Welfare, 2013). It is the second most common cause of death in women (Makhoul I., 2006). The advent of screening programmes and improved imaging has led to earlier breast cancer detection. Combined with more advanced oncoplastic surgical techniques and adjuvant therapies, survival rates have increased in recent years. The AIHW reported an increase in five-year survival of 72 per cent between 1982-1987, to 89.4 per cent between 2006-2010 (Australian Institue of Health and Welfare, 2013). In a woman who has been diagnosed with a tumour between 0.1cm-1cm diameters, the five-year relative survival is 98.2 per cent. For tumours 1.1cm-1.5cm it's 94.7 per cent, 1.6cm-1.9cm 93 per cent, 2cm-2.9cm 87.9 per cent and 73.1 per cent for tumours greater than 30mm (Australian Institue of Health and Welfare, 2013).

The mainstay of treatment for early breast cancer is breast conserving surgery or total mastectomy, to excise the primary tumour with or without lymph node

dissection. The surgical defect that remains depends on whether the patient has had breast conserving surgery or total mastectomy. Depending on the extent of disease in the breast, breast conserving surgery defects can create a substantial defect in shape and appearance yielding poor cosmetic results. Total mastectomy leaves a flat chest in the breast area, with a longitudinal scar which is usually insensate. Bilateral total mastectomy will leave long scars on both sides of the chest with complete loss of the breast mounds. Therefore, breast cancer surgery can have long term effects on women's quality of life with reduced self-esteem, diminished psychological well-being, a feeling of "imbalance" and restricted freedom of dress.

Breast reconstruction procedures are increasingly sought by patients to ameliorate psychosocial impact of breast cancer surgery and improve aesthetics. Current options for reconstruction of mastectomy defects can be broadly divided into artificial implant-based reconstructions and large mycocutaneous pedicled or micro-vascular free flaps. Free Transverse Rectus Abdominus Myocutaneous (TRAM) flap and Deep Inferior Epigastric Perforator (DIEP) procedures involve micro surgery and can take 8-10 hours. Flap surgery involves transferring a whole block of skin, fat and sometimes muscle from a distant part of the body to the mastectomy defect. It requires long incisions in the skin and interference with the normal anatomy of the muscles, nerves and blood vessels. It leaves long permanent scars and often some muscle weakness. Patients are required to stay in hospital for 5-7 days so the wounds and flaps can be reviewed. Although autologous flap surgery has improved in terms of reliability, there is still an incidence of flap failure between 2-5 per cent. Flap failure results in serious consequences for patients, including repeat surgery. Recovery time from these procedures is six to eight weeks.

Silicone implant-based reconstruction has the advantage of reduced surgical

time and an ability to produce breast reconstructions of a variety of sizes, independently of the volume of donor tissue available. Use of silicone prostheses can, however be associate with a variety of complications including capsular contracture, prosthetic infection or implant rupture. Once silicone is implanted into the body a fibrous capsule forms around the implant. Capsular contracture results from tightening of the collagen fibres in the fibrous capsule causing distortion of the implant, shape deformity and pain. This requires surgical intervention such as capsulotomy or capsulectomy, with replacement of implants. Ruptures of implants have resulted in multiple controversies leading to high levels of patient anxiety. Recent recall of PIP (Poly Implant Prothèse) implants because of a high frequency of rupture and undetermined contents of the implant was widely publicised.(Health, 2013) As a result, some patients are adamant they do not want to have implants as a reconstructive option. A less common but more concerning complication is the 56 reported cases of Anaplastic Large Cell Lymphoma (ALCL) in women with silicone implants in Australia. (Therapeutic Goods Administration (TGA), https://www.tga.gov.au/alert/breast-implants-and-anaplastic-large-cell-lymphoma). The lymphoma was found to have originated in the capsule around the implant. (Buchholz, 2009)

One of the reconstructive surgical options available for women who have undergone mastectomy for breast cancer is autologous fat transfer, otherwise described as lipo-modelling, lipo-sculpting or fat grafting. Fat grafting is a process that involves the removal of fat from one area of the body and re-injecting it into another part of the body. Autologous fat transfer dates back to 1895 with a breast reconstruction using a large lipoma by Czerny (A., 1895). Prior to this Neuber was using fat autografts for facial depression correction (F., 1893). Bartlett W in 1917

published the first case series of six patients who had en bloc fat excised from the abdomen, which was then ‘stuffed’ into the breast defect.(Bartlett, 1917) Although basic in his application, the patients reported satisfaction and he only noted 1/10 of graft loss. Pioneered in the nineteenth century, this procedure was not widely used as a therapy, due to early concerns regarding its efficacy and safety. Bircoll's papers in 1987 explained that after transfer of fat there was fat necrosis and generation of microcalcifications and cysts (Bircoll, 1987, Bircoll M, 1987). These findings were met with concern from the surgical community because it was thought microcalcifications could interfere with radiological imaging for breast cancer. That same year the American Society of Plastic and Reconstructive Surgeons (ASPRS) Ad-Hoc Committee on New Procedures concluded that the procedure could lead to high false positive results and deplored its use in breast cancer patients (Fredericks S, 1987). The basis of this decision was the findings of calcification on mammography and long term volume loss of the graft, through either necrosis or scar formation. The ASPRS decision did not have statistically significant data or scientific research to support their conclusion but rather relied on panel member specialist opinion. Veber’s paper on radiological finding after fat grafting did demonstrate abnormal findings which include macro and micro calcifications, cysts, and evidence of tissue remodelling (Veber et al., 2011). However, with improvements of imaging in the 21st century, there is also evidence that the appearance of fat necrosis calcification is distinctly discernible from malignancy (Australian Institue of Health and Welfare, 2013). The ASPRS Ad-Hoc Committee have since opined that although fat grafting is feasible further high quality studies are needed (Fredericks S, 1987). Their 2012 guiding principles statement outlined that although previously perceived to be experimental, in instances of total mastectomy where there is no native breast tissue there is increasing evidence supporting its safety and efficacy (Surgeons, 2012).

Furthermore fat grafting is an effective option in breast reconstruction following mastectomy, as it has demonstrated moderate to significant aesthetic improvement. In addition fat grafting is a viable option for improving skin quality post irradiation.

A recent paper reported on the trends in autologous fat grafting to the breast as a national survey of American Plastic Surgeons (Kling et al., 2013). Their questionnaire study of 2584 plastic surgeons revealed that sixty two per cent were using autologous fat transfer for reconstructive breast surgery. However, clinicians should employ the available information while remaining cognizant of newer, evidence based findings. In order to understand the possible adverse effects of fat grafting to the breast in women who have had breast cancer surgery an understanding of the role of adipose derived stem cells warrants exploration.

1.2 Basic Science

1.2.1 Adipocytes role in oncogenesis

Adipose tissue consists of mature adipocytes, pre adipocytes (10% of all cells), fibroblasts, mesenchymal stem cells, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages, lymphocytes and adipose derived stem cells (ADSCs) (Brown et al., 2010). Of these cells pre adipocytes determine graft viability as they seem more resistant to avascular conditions. Post grafting mature adipocytes either die, survive or undergo differentiation. In order for pre adipocytes to mature into adipocytes, there are several key genes which need to be expressed including lipoprotein lipase, peroxisome proliferator-activated receptor and enhancer binding proteins (Vona-Davis and Rose, 2007). In theory targeting these genes for over expression after fat graft transfer leads to improved maturation of pre adipocytes. A method of doing this has not yet been designed.

Adipose derived stem cells (ADSCs) have been theorised to have the following characteristics: multipotent differentiation capability, release of angiogenic growth factors which could assist with neovascularisation and they may proliferate after transplantation(A, 2009). Therefore, concentrating ADSC's and supplementing fat grafts should lead to improved microvascular supply of the transplanted fat improving outcomes (Eto H, 2011). ADSCs are easily harvested with liposuction and concentrated through a process of collagenase breakdown and washing (Sterodimas et al., 2009)

This method has been explored by Eto et al. who described cell assisted lipotransfer (CAL) for cosmetic breast augmentation (Eto H, 2011). They demonstrated improvements in volume at 6 months follow up, compared to volume loss reported in other studies (Zheng DN, 2008, Coleman, 2007). The role of ADSCs

in breast cancer patients is an area of ongoing research as outlined in the ASPS statement on Stem Cells and Fat Grafting (Eaves et al., 2012). A substantial body of clinical data still needs to be obtained.

1.2.2 Graft Survival theory vs host replacement theory

The two key theories on the survival of fat graft after transfer are the graft survival theory and the host replacement theory. Eto's team observed that the outcomes of clinical fat grafting were variable (Eto H, 2011). They investigated the cellular components of fat by comparing stored lipoaspirate with cultured cells. They used organ-cultured adipose tissue which was taken from the groin fat pad then placed into the scalp of the same mice (Eto H, 2011). The transferred fat tissue was then stained at intervals over several days. They found that in the first few days it was mostly the adipose derived stem cells that remained viable. They showed that most adipocytes begin to die in the ischaemic environment on day 1. The adipocytes that survived were within 300 μm of the edge of the surrounding tissue suggesting that they received some nutrient supply for them to remain viable. After day 3, the adipose derived stem cells seemed to become more viable suggesting that there was promotion of regeneration. This occurred until day 7 demonstrating a period of active remodeling and survival of a non vascularised adipose graft. Eto et. al. then suggested that the clinical application may be that if fat is taken from one part of the body, the graft survives rather than the body replacing the fat graft with some other substrate, for example, a fibrovascular scaffold. It was therefore hypothesized that the use of autologous fat grafting may be efficacious when used for injection into other parts of the body.

1.2.3 *In vitro* and *In vivo* Studies

In vitro studies on the biology of fat graft post transplantation show that adipose derived stromal cells survive in ischaemic conditions for 72 hours compared with adipocytes, which die in 24 hours (Eto H, 2011). In the first 48 hours post grafting direct diffusion of nutrients maintain the graft from the surrounding tissue bed (Mizuno and Hyakusoku, 2010). After 48 hours three distinct zones are observed within the graft - from the periphery to the centre of the graft: surviving area (viable adipocytes), regenerating area (adipocytes died whilst ADSCs survived) and the necrotic area (all cells died). Brown et. al. discussed the interest in properties of adipose derived stem cells which in vitro have shown multipotent capabilities by differentiating into either adipocytes, osteoblasts, and chondroblasts (Brown et al., 2010). Theories as to the origin of ADSCs are that they arise from pericytes (perivascular mural cells) around blood vessels or a sub population of fibroblasts that reside within adipose tissue.

The role of adipose tissue in cancer development in experimental studies has shown that through endocrine and paracrine activity adipose tissue resident progenitor cells produce growth factors which could act on cancer cells (Petit JY, 2011, Vona-Davis and Rose, 2007). Another theory by the same author is the cell signalling pathway of leptin on oestrogen dependent and independent cancer cell types. Lohsiriwat experimental studies show that preadipocyte and progenitor cells can stimulate angiogenesis and cell growth(Lohsiriwat V, 2011). Perrot et al. implicated mesenchymal stem cells in osteosarcoma recurrence(Perrot et al., 2010). Human mesenchymal stem cells were concentrated and injected into mice along with POS-1 osteosarcoma cells. The control group was injected with only POS-1. In vitro they found increase rates of tumour growth with the MSC group.

Cell assisted transfer of fat graft and ADSCs has been conducted in immunodeficient mice(Eto H, 2011). Fat was grafted alone versus with ADSCs to compare microvasculature and overall survival of fat. The group with ADSC showed more prominent microvasculature. Clinically there are no randomised control trials to date which demonstrate feasibility of cell assisted transfer fat.

Guerrerosanto et al. looked at the long term survival of fat grafts in rats. They showed good survival of fat once transplanted in to muscle and emphasized the point that the fat graft should be thin and placed in a high nutrient, well vascularized host bed area(Guerroresantos J, 1996).

1.3 Clinical Research

1.3.1 Safety

Thus far, from the European experience there has not been found to be any greater risk of cancer recurrence in fat transferred patients compared with the general population of breast cancer patients. Claro et al. reported a recurrence rate of 2.3% (14 women of 616 patients) who had undergone mastectomy, whilst another group reported a rate of 7%(Claro Jr, 2012, Rigotti G., 2010). One study by Delay after performing 880 cosmetic and reconstructive procedures, over a 10 year period, reported no increase in incidence of breast cancer (Delay et al., 2009a). These earlier papers defied the dictate for extreme caution when considering fat grafting for breast cancer patients and produced favourable results refuting earlier concerns. A more recent multi-centre trial of 646 lipofilling procedures for breast conserving surgery or total mastectomy patients, with an average time between cancer surgery and lipofilling of 39.7 months reported a complication rate of 2% for liponecrosis and a recurrence rate of 5.6% (Petit JY, 2011). Seth et al. compared 99 fat grafting patients 18.3 months (mean) post mastectomy and found no increase in local recurrence compared with 1112 women of same demographic, operative, oncologic and post-operative factors (Seth et al., 2012). No increase in incidence of recurrent tumour in post mastectomy patients was found by Delay, Sarfati, Petit or Rigotti .(Delay et al., 2009a, Sarfati et al., 2011, Petit JY, 2011, Rigotti G., 2010).

It has been argued that because of their vascularity, autologous flaps should theoretically have a significantly greater oncologic potential than free fat grafts, if in fact fat stem cells can stimulate cancer recurrence (Delay et al., 2009a). This has not been borne out in clinical experience of breast cancer reconstruction. A recent paper published by Casey et al., a research group at the Mayo Clinic reported on rates of

recurrence in flaps (TRAM, MS-TRAM, DIEP, SIEA) used for breast reconstruction(Casey et al., 2013). Recurrent carcinomas were diagnosed in 13 reconstructed breasts which was 3.6% of reconstructed breasts (n=365). Mean time frame to recurrence was 24 months.

When reviewing the papers in the twenty-first century, radiological findings suggest that there is no difficulty in diagnosing breast cancer during mammographic screening (Rubin et al., 2012, Fraser et al., 2011, Claro Jr, 2012, Carvajal J., 2008). There has also been a recent studies showing that finding are similar between breast reduction and breast fat grafting groups (Fraser et al., 2011). Currently, there remains a paucity of evidence when it comes to oncologic potential of fat transfer. However, with the transfer of fat there is a new environment created for surrounding cells, including quiescent cancer cells. The possibility of "fuelling" dormant breast cancer cells through progenitor cell secretions from pre-adipocytes was outlined by Lohsiriwat et al. (Lohsiriwat V, 2011). There were two cases demonstrated by Coleman and Sabreiro where patients without a history of breast cancer were diagnosed post fat grafting (Coleman, 2007). However, breast cancer is common in the overall population.

A recent case report by Perrot et al. described the recurrence of osteosarcoma 18 months after a lipofilling procedure. Interestingly, the primary osteosarcoma had been removed 13 years prior(Perrot et al., 2010). As late local recurrence is extremely rare, this raises a question regarding effect of grafted adipocytes on dormant cancer cells. Missana conducted a case series of breast conserving surgery patients who had undergone breast conserving surgery and found there was no recurrence on MRI for a mean follow-up period of 11.7 months. (Missana MC, 2007).

Some determinants of local recurrences risk were explored by Petit et al. suggesting an association with women who were less than 50 years of age had high grade neoplasia and had undergone quadrantectomy (Petit et al., 2012). The most significant finding was of the relationship between recurrence and the Ki-67 level being greater than 14. Unfortunately, this study was limited by its retrospective nature, small sample size and variability of breast cancer treatments. However, their findings of increased recurrence rates of up to 18% in high risk patients who have had fat grafting is difficult to ignore. His earlier work had suggested that breast conserving surgey/quadrantectomy and mastectomy patients had no increase in incidence of cancer recurrence (Petit et al., 1998).

Two retrospective matched case-control studies by Gale et.al. and Kronowitz et.al. explored the potential link between autologous fat grafting and breast cancer (Gale et al., 2015, Kronowitz et al., 2016). Gale et. al. compared 211 women who had undergone fat grafting post breast cancer surgery with a control group of 422 women. Kronowitz et. al. had a larger study involving 719 women who had lipofilling post breast cancer compared with 670 controls. Both level 3b studies demonstrated no increase of risk of locoregional recurrence rates in the setting of autologous fat grafting post breast cancer surgery. Interestingly, Kronowitz et.al. noted an increase in incidence of recurrence in women receiving hormonal therapy as adjuvant therapy (Kronowitz et al., 2016). This suggests there may be additional factors other than adipose derived stem cells that may predispose to cancer recurrence. This would need to be explored further.

There is paucity of good quality clinical research on autologous fat grafting in breast conserving surgery and total mastectomy patients. The study designs of most papers are retrospective cohort studies involving either a single institution or single

surgeon's experience. Some papers are case series with small sample sizes, or case reports which can be regarded as anecdotal evidence. Systematically reviewing the literature, there are approximately 50 clinical studies on this specific patient group and these manuscripts are discussed further in Chapter 2 (Spear SL, 2005, Delay G., 2009, Rigotti G., 2010, Khouri R.K., 2012, Coleman, 2007, Ribuffo et al., 2011, Rietjens et al., 2011, Salgarello et al., 2011, Uda et al., 2014, Bonomi et al., 2013, de Heras Ciechomski et al., 2012, Losken et al., 2011, Seth et al., 2012, Sinna et al., 2010, Ihrai et al., 2013, Kanchwala et al., 2009, Gosset J, 2008, Hammer-Hansen et al., 2015, Petit JY, 2011, Petit et al., 2012, IIllouz YG, 2009, Delay et al., 2009a, Pierrefeu-Lagrange AC, 2006, Delaporte et al., 2009, Babovic, 2010, de Blacam et al., 2011, Serra-Renom et al., 2011, Panettiere et al., 2011, Panettiere et al., 2009, Missana MC, 2007, Petit et al., 1998, Beck M, 2011, Choi et al., 2013, Rigotti G., 2005). The majority of papers include fat grafting for breast reconstruction post breast conserving surgery or total mastectomy, while others use fat grafting in conjunction with autologous flap reconstruction, or artificial implant surgery. The majority of papers reporting on a group of patients who had undergone fat grafting had subsets of patient groups, e.g. autologous flap reconstruction, implants reconstruction, and revision after lumpectomy surgery. One paper did not state whether autologous fat grafting was used for cosmesis, reconstruction or both (Carvajal J., 2008). As there are differing methods of reporting on complications, evaluation of outcomes, statistical analysis, inclusion and exclusion criteria and patients are not well matched it is difficult to form comparisons. Lack of generalisability of methodology makes it impossible to conduct a meta-analysis of the literature. Four authors have attempted to conduct a formal systematic review and meta-analysis however there are obvious limitations in these articles relating to the low level of evidence cohort studies that were used without control groups

(Groen et al., 2016, Krastev et al., 2013, Leopardi D, 2010, Waked et al., 2017). Waked et. al. explored 18 clinical studies which demonstrated loco-regional recurrence rates of between 0- 1.62% for breast conserving surgeries and 0-3.9% for total mastectomy. Unfortunately the search period of the study was from 2014-2016 so this may not be reflective of all clinical studies nor were books and other languages included in the search, introducing publication bias (Waked et al., 2017).

Generally, in the articles explored to date, author bias could be found with declaration of sponsorship from a commercial fat grafting group (Cytori). Other limitations were small study numbers or evidence of poor statistical analysis (Khouri R.K., 2012). Coleman and Delaporte did not have quantitative measurement of their results (Delaporte et al., 2009, Coleman S.R., 1995). More recent studies like that of Silva-Vergara who have conducted 319 lipofilling procedures in 132 mastectomy and 63 breast conserving surgery patients has demonstrated promise with a loco-regional relapse of 3.1 or 1.08% per year which is comparable to the rates of recurrence in a normal population of women who have undergone breast cancer surgery. Their rate of 8.3% for liponecrosis and oil cyst rate was slightly higher than that found in the literature search in Chapter 2 of this thesis. This may be related to the fact that their technique was not Coleman's technique and involved centrifugation at a high rate of 2000 rpm (Silva-Vergara et al., 2016).

Notwithstanding the short comings of these papers, they demonstrated feasibility for efficacy and safety of fat grafting, refuting earlier claims. One consistency in these papers was the use of the Coleman technique as a method of fat harvest and fat grafting. The majority of papers conclude that there needs to be further higher quality studies, including a recent statement from ASPRS. Although the ASPS guidelines state that fat grafting to the post mastectomy breast does not

delay breast cancer detection or increase breast cancer recurrence.(Surgeons, 2012) There is an obvious need for further randomised control trials.

The purpose of Chapter 2 of this thesis is to outline all manuscripts in the current literature for all women who have undergone breast cancer surgery and then autologous fat grafting. It also assesses the quality of all manuscripts using the STROBE statement as an independent review tool (Ohm, 1827).

1.4 Radiology

Difficulty in interpretation of digital mammography post autologous fat transfer has previously been documented (Fredericks S, 1987). Consequently, radiological caveats include formation of palpable breast masses, transferred fat obstructing adequate view of breast parenchyma, compression of the parenchyma and formation of calcifications which can mimic malignancy (Cheung et al., 2000, Pulagam SR). These concerns are not isolated to autologous fat transfer with the Coleman techniques, but with reconstruction using flaps, eg. Latissimus Dorsi Flaps. Pierrefeu-Lagrange reported on thirty cases retrospectively, four of which had microcalcifications (Pierrefeu-Lagrange AC, 2006). Chan et al. has described such microcalcifications as round, punctate, dystrophic, clustered, curvilinear and 'ring like'(Chan CW, 2008). Fat necrosis results in fatty acid release and combines with calcium to form calcifications. These have also been depicted on mammography as curvilinear egg shell shaped calcifications or calcifying oil cyst (Pulagam SR). In Carvajal's series of 20 patients, the microcalcification were reported as BI-RADS II lesions, i.e. benign findings (Carvajal J., 2008).

As fat graft is avascular, after transfer it may undergo necrosis presenting clinically as a mass sometimes associated with skin or nipple retraction, indistinguishable clinically from malignancy. Masses formed may be calcified, non-calcified and appear cystic (Pulagam SR). On ultrasound there are non-diagnostic areas of altered echo texture described as either complex cysts, hypoechoic lesions with mild acoustic enhancement, hypoechoic lesion with echogenic anterior rim and dense acoustic shadowing, and tiny cystic structures (Cheung et al., 2000, Pulagam SR). Fine needle aspiration will reveal birefringent spicules easily distinguishable from malignant cells (Cheung et al., 2000).

Advances in imaging have enabled differentiation between benign versus malignant calcification, allowing earlier detection of recurrence. Missana undertook T1 and T2 weighted contrast MRI pre op then 3 months post op looking for evidence of cytosteatonecrosis and "evolution of graft"(Missana MC, 2007). Contrast will capture DCIS and enables early detection of recurrences by revealing neoangiogenisis. T1 weighted sequences are better for analysing adipose tissue as normal breast adipose tissue has higher enhancement. Cystosteatonecrotic lesions are characterised by hypo intense zone surrounded by hyper intense ring (Missana MC, 2007). Gadolinium enhances recurrences but not cystosteatonecrosis. Local recurrences are usually identified by irregular microcalcifications or suspicious mass near a scar (BIRADS 4 or 5). (33) Once the grafted fat has taken, it is indistinguishable from the surround breast tissue (Missana MC, 2007).

In contrast to fat transfer, Silicone implants interfere with mammography by the capsule that is created(Pulagam SR). The capsule obscures view due to its high roentgen density resulting in late recurrence detection(Pulagam SR). In earlier papers, transplanted fat was theorised to compress breast parenchyma distorting breast architecture but with advances in imaging this has been abrogated. Calcification of transplanted fat is a slow process which can be monitored with appropriate imaging (Hughes et al., 2009).

Fraser reported on ten papers spanning 2,000 cases and reported no difficulty in diagnosing breast cancer (Fraser et al., 2011). Unfortunately, three authors were sponsored by Cytori, the commercial fat grafting group. A newer study by Rubin compared mammography for breast reduction patients versus fat grafting for augmentation (Rubin et al., 2012). Eight radiologists were double blinded and

independently reported on fifty patients MMGs. There were higher recordings for masses which required biopsy in the reduction group, 25 cases versus six suggesting that fat grafting had no higher risk of producing confusing mammographic appearances than this common and well accepted procedure. Limitations of this study were low number of patients that were not age matched.

The gold standard for breast reconstruction is autologous flap surgery. In Casey et al.'s flap study mentioned previously, there were 66 breasts (18%) of 365 breast which had fat necrosis and subsequent formation of masses which needed further investigation (Casey et al., 2013). Other breast reconstruction papers had similar reporting of radiographic changes (Pierrefeu-Lagrange AC, 2006, Coleman, 2007, Gosset J, 2008, Zheng DN, 2008, Veber et al., 2011).

1.5 Autologous Fat Grafting

1.5.1 Versus Conventional Reconstructive Operations

The Transverse Rectus Abdominis Myocutaneous (TRAM) flap involves taking some of the rectus abdominus muscle with overlying skin and fat as either a pedicled or a free flap. A free flap requires microsurgery to re-establish its blood flow at the donor site in the breast. Latissimus dorsi flap reconstruction involves taking skin from the back which overlies the latissimus dorsi muscle. The muscle is dissected free from its origins and moved on a pedicle under the skin of the axilla to the front of the chest. The benefit of a pedicled TRAM and LD is that the surgeon does not need micro surgical experience and the benefit of a free TRAM is that less muscle needs to be harvested from the abdomen, leading to less post-operative muscle weakness, and there is a lower incidence of partial flap failure.

Perforator flaps like the DIEP and SIEA flaps require microsurgical techniques and are considered the 'gold standard' for breast reconstruction. The disadvantage is the time it takes to dissect the vascular pedicle from the rectus muscle and the need for microsurgery.

The disadvantages of any flap reconstruction procedure is the long surgery times, long hospital stay and associated donor site morbidity including herniation. The large scar on the back has associated morbidity of infection, bleeding and poor wound healing. Fat necrosis rate are zero with LD, 15% in the free TRAM and 47% in the pedicled TRAM, 13.4% for DIEP flaps and 5.7% for the SIEA flap (Casey et al., 2013). Bilateral DIEP flaps can take up to 12 hours in duration as care is needed not to damage the inferior epigastric vessels as they are being traced through the rectus abdominis combined with the time it takes for microsurgical anastomosis.

One of the key advantages of fat grafting is that it is autologous. As the fat is harvested from the same individual it can avoids the need for artificial implants. In addition, there is lower donor site and recipient site morbidity with smaller incisions and reduced infection and haematoma rates. The mean time for the procedure is 115 minutes (range 60-165 min)(Missana MC, 2007). The major disadvantage is with fat resorption and unpredictability of results. The reporting of fat necrosis will be discussed in section 1.4.

With respect to technical difficulty, risk of complications and patient recovery autologous fat grafting is appearing superior. With the exception of the pedicle TRAM flap, fat necrosis is still a greater problem when compared to flap reconstruction, and reducing the amount of fat necrosis in fat grafting procedures is a focus of current research. Despite the risk of fat necrosis, from an aesthetic perspective, autologous fat grafting has been shown to be a useful adjunct to various aspects of breast reconstruction. This includes enhancing mound volume, contour, superomedial fullness and patient satisfaction (de Blacam et al., 2011, Delay et al., 2009b, Kanchwala et al., 2009, Losken et al., 2011, Ribuffo et al., 2011, Sarfati et al., 2011, Serra-Renom et al., 2011, Sinna et al., 2010, Panettiere et al., 2011). Cosmesis will be discussed further in section 1.5. Fat grafting will not preclude patients from further procedure, whereas in cases of previous Abdominoplasty, DIEP flap reconstruction is contraindicated. And in instances of recurrences, breast cancer surgery is uncomplicated if patients have had fat grafting(Missana MC, 2007).

1.5.2 Indications

The use of fat grafting extends to many areas of the body. For otolaryngology and recontouring of the vocal cord folds, filling of bone defects in orthopaedics, in neurosurgery for repairing CSF leaks and in colorectal surgery as an injection for

sphincter incompetence(Chan CW, 2008). Coleman described a technique in 1995 for facial recontouring that has since been used for the breast. Indications include micromastia, tuberous breasts, Poland Syndrome, post mastectomy pain syndrome, breast conserving surgery (repair of contour defects +/- alleviation of pain), adjunct to post mastectomy autologous or implant based reconstruction (with or without post mastectomy radiotherapy); total breast reconstruction using AFG; AFG for breast augmentation, AFG for rejuvenation of radiotherapy damaged tissue (Petit JY, 2011, Coleman, 2006, Riggio et al., 2013). These subgroups of patients and clinical studies are described in more detail in Chapter 2.

In 2009 Delaporte and Delay et al. showed a retrospective case series on 15 patients who had undergone flap reconstruction post total mastectomy (Delaporte et al., 2009). During a follow up period of 28 months these patients required treatment with fat grafting for contour deformities. There was no objective measurement of results but two thirds of patients stated they were satisfied and external judges assessed aesthetics as being very good. de Blacam et al. specifically identified its use to correct breast deformities in the superomedial area of the reconstructed breast called the “step off”(de Blacam et al., 2011). Missana et al. used it to mask unsightly folds post silicone implantation and to obtain a harmonious gradient with the reconstructed segment after autologous flap reconstruction (Missana MC, 2007).

Caviggioli et al. demonstrated the utility of autologous fat transfer in post mastectomy pain syndrome defined as chronic neuropathic pain lasting longer than 3 months in the chest, axilla or upper arm area post mastectomy (Caviggioli et al., 2011). A total of 63 patients who had previously undergone mastectomy, axillary clearance and radiotherapy were enrolled. Injecting fat (55cc) into the dermo hypodermal junction, they found statistically significant evidence in a reduction of

pain scores on a visual analogue scale. Twenty-eight patients were able to cease their analgesic therapy at 13 months follow up. The proposed hypothesis of action was of remodelling of scar tissue through angiogenesis in areas which were causing nerve entrapment.

Autologous fat grafting can be used during a corrective procedure in patients who experience capsular contracture (described in section 1.1) from silicone implants. If patients present with Baker grade 3 or 4 contracture where the breast is firm and appears abnormal or is painful with deformation, they will normally require a capsulotomy with removal of the capsule. Missana described either surgically removing the capsule, down grading the size of the implant then supplementing it with fat graft (Missana MC, 2007), or leaving out the implant and filling the defect with fat graft. Although early results appear good, the outcomes of this technique need to be explored further.

Fat grafting has also been used in burns scars Klinger et al. grafted purified fat graft in three patients with severe facial burns scars. Clinically they obtained considerable improvement in skin texture and thickness with clinical reduction in pain. Biopsies were taken of the scar tissue pre and post autologous fat transfer which showed improved local hypervascularity (Klinger et al., 2008).

1.5.3 Patient Selection

Autologous fat transfer should be administered with caution in patients who are at high risk of breast cancer (Surgeons, 2012). It is likely that breast cancer patients with mutations in the BRCA 1 or 2 genes, would have higher risk of tumour recurrence(Pearl et al., 2012). Incomplete resection on histology of a breast conserving surgery specimens also pre-disposes patients to higher risk of recurrent tumour. The breast tissue of patients who have had a high histological grade breast

cancer may be more susceptible to stimulation from imported fat cells. Casey et al.'s retrospective study on mastectomy and fat grafting reveal recurrence rates of 0-5.3% in patients with previous DCIS and for invasive carcinoma 0-9.5% (Casey et al., 2013). In the setting of nodal disease at the time of operative intervention, the risk of local recurrence is elevated to 19-27%. High risk patients were classified as being from a younger age group, having larger tumour sizes, having higher tumour grades, narrower margin width (<1cm), multi-centricity and lymphovascular invasion (LVI). Although these rates don't preclude patients from reconstructive procedures, masses which present post fat grafting should be thoroughly investigated in the presence of any these risk factors.

The literature does not explore whether associated co-morbidities result in worse outcomes, e.g. smoking, vascular disease or diabetes. As fat is relatively avascular, patients who have had radiotherapy were previously considered to be poor candidates as post radiated tissues are poorly vascularised and ischaemic. However, because of the high risk of failure of artificial implant reconstruction in these patients, fat grafting has been attempted as an alternative. Rigotti et al. explained that the effect of radiotherapy on skin and subcutaneous tissue can last years in a progressive self-maintaining pathology which can be graded (Rigotti G., 2005). Using a LENT-SOMA classification of radiotherapy damaged breast tissues (Grade 1 being present skin damage, Grade 2 of having associated symptoms, Grade 3 involving severe symptoms and Grade 4 being irreversible functional damage) he demonstrated the effect of ADSC therapy on reversing ischaemic damage through improvement of their LENT-SOMA scores. Improvements in scarring post fat grafting has been commented on by other authors (de Blacam et al., 2011, Losken et al., 2011, Panettiere et al., 2009, Serra-Renom et al., 2011).

1.5.4 Techniques of AFT

Coleman was the first to establish a method for autologous fat transfer (AFT) involving harvesting fat with atraumatic liposuction, injection of purified adipocytes with centrifugation and then injection (Coleman, 1997). His technique was described as “structural fat grafting” where smaller quantities of fat with higher surface area to volume ratio yielding improved graft retention rates. However, too small a graft would be resorbed. Unfortunately, in this paper there was no reference to benefits and limitations of each step and no quantitative measure of outcomes. Multiple other minor variations in methods have been documented with use of centrifuge and additives like insulin, platelet rich plasma, endogenous stem cells, and thyroid hormone. The Lipivage system is a single unit which uses disposable cannulae. Fat graft harvest in undertaken under low suction, without centrifugation. There is no difference in outcomes when comparing the Lipivage system and the Coleman technique with centrifugation (Ferguson et al., 2008, Pu L L.Q., 2006). Alternative techniques to the Coleman technique are discussed in Chapter 2 (2.4.10).

In the recent survey of 2584 American Plastic Surgeons half recorded poor retention rates of fat graft or unpredictable results as being the greatest obstacles faced with this procedure (Kling et al., 2013). Surgeons continue to alter technique in order to overcome the loss of fat graft. Illouz et al. conducted multiple session liposuction (IIllouz YG, 2009). His preparation of fat prior to harvest was to allow it to stand or decant for 10-15 min prior to injecting it subcutaneously and intra-glandular. The paper discusses not injecting into the retro glandular space which may not have enough vascularisation for the fat graft to survive. The authors suggests that injecting into an area which already has sufficient blood supply, e.g. subcutaneously

or intra glandular, would permit better outcomes which accords with Guerrerosanto's in vivo studies (Guerroresantos J, 1996). Another theory for improved outcomes in this paper was of creating "pearls" of adipose tissue in a drop-to-drop manner increasing the chance of adipocyte survival. This may be because of improved nutrient supply from surrounding tissues and therefore improved viability of adipocytes.

Serra-Renom conducted whole breast reconstruction in 8 patients and planned three stages for injection for total graft volume of 350mls (Serra-Renom et al., 2011). His team did this with the use of anchoring stitches in the inframammary fold and injection of 150mls of fat in Step 1. After 3 months, Step 2 is to inject a further 150mls, puckering sutures in the inframammary fold to maintain shape. The final stage involves 100mls of fat graft injection, and a 'cone shaped pexia'.

1.6 External Mechanical Expansion of the Breast or Breast Skin Envelope Prior to Fat Grafting.

Another modality to increase graft retention rates is the Breast Reconstruction and Augmentation, Volume and Aesthetic (BRAVA®) external expander device (Khouri R.K., 2012). It is an external tissue expander that works by exerting an external vacuum pressure, or isotropic distraction force on the breast, or mastectomy skin flaps. In doing this, it is theorised to have four effects on the breast: mechanical expansion stimulating angiogenic cytokines, increased physiological space reducing crowding of breast parenchymal tissue and filling pressure, up-regulation of growth factors, and creating muscle tissue with high capillary density. The BRAVA® is worn like a brassiere. Within its fabric there are two semi rigid polyurethane domes that are placed around the interface with the skin through silicone gel-filled donut bladders. The bladders form an air tight seal, help dissipate pressure, and shear forces. A small battery-operated, microchip controlled mini-pump maintains 20mm/Hg of negative pressure inside the dome. It exerts an isotropic distractive force to the breast. Anticipated increase in size of the breast is one "cup-size" in those with normal breast tissue. Preliminary tolerance of the device is required to establish whether the device can be worn for longer periods of time. This is usually carried out in the outpatient setting with a 20-minute trial. Use of the BRAVA® device should be painless. If painful, women are asked to remove the device. The BRAVA® is requested to be worn for 4 weeks prior to the autologous fat transfer procedure for 10 hours per day (Khouri R.K., 2012). Post-operative expansion with the BRAVA® device has been theorised to "stent the graft" and decrease the amount of contraction of the skin on underlying graft adipocytes, improving viability (Khouri R.K., 2012). Patients need follow up to establish a satisfactory result pending repeat procedures as determined Missana who had 14.86% (11 of 74) of his

patients requiring repeat procedures (Missana MC, 2007). This device needs further objective quantifiable measure of its outcomes which is addressed in Chapter 5.

1.7 Donor Sites for Harvest of Fat

Patients should be counselled pre operatively that they may require more than one session to achieve adequate reconstructed breast volume (de Blacam et al., 2011, Kanchwala et al., 2009, Sinna et al., 2010). Vecchio and Fichadia suggest a total mastectomy patient usually requires 3-4 sessions of fat transfer spaced four months apart (Fichadia, 2012). In those patients with radiation, an increased number of sessions may be required, in the order of 4-5 times. Illouz conducted multi session fat grafting with 3 month intervals in order to reduce the complications associated with injecting excessive amounts of fat (IIllouz YG, 2009). Illouz suggests this is due to fat necrosis and cyst formation.

Marking is done preoperatively and clinically some donor sites are better than others for fat harvesting. Outer thigh versus inner thigh versus lower abdomen. However, scientifically, there is no difference in flank, lower abdomen, medial knee and thigh for the ability to produce proliferating cells in culture (Rohrich et al., 2004). Outer and inner thigh and the flank seem to appear resistant to weight fluctuation (Markey and Glogau, 2000).

Donor sites are planned prior to anaesthesia with the patient standing (Coleman S.R., 1995). Pre-operative considerations for autologous fat grafting include the use of antibiotics. Delay did not use antibiotics for lipomodelling and this is likely to be subject to surgeon preference. Chan et al. explained that the bore of the injecting cannula is important in minimizing fat cell trauma (Chan CW, 2008).

Adanali showed that viability of adipocytes decrease with increased suction, excessive handling, refrigeration or major trauma during tissue collection or processing (Chan CW, 2008). Khouri creating his own suction with a Khouri cannula which is hand driven with suction pressure created by withdrawing a plunger (Khouri R.K., 2012).

Operatively, owing to the large quantities of fat required patients usually have a general anaesthetic. Local anaesthetic is only used for smaller revision with smaller volumes of fat grafting (e.g. <50mls). With regard to local tumescent infiltration some surgeons use adrenaline for its vasoactive properties, decreasing blood in the liposuctioned fat. Others state that adrenalin will decrease fat graft viability by constricting blood vessels (Chan CW, 2008).

Determining the amount of fat to harvest depends on the indication e.g. cosmetic, defect correction, and breast conserving surgery or total mastectomy breast reconstruction. Volume can be added to autologous flap reconstruction but the amount required will be less than for whole breast reconstruction. Low volume is defined as less than 100mls and high volumes are greater than 100mls. In Choi et al.'s recent paper he reviewed different volumes of fat graft and their maintenance over time using a Vectra 3D photographic equipment (Choi et al., 2013). He concluded that larger volumes injected achieve volume stabilisation quicker. This is in keeping with Delay's suggestion that you should over correct volumes to account for reabsorption (Delay et al., 2009a). Missana used an average of 75mls for breast conserving surgery and 142mls as adjunct with flap reconstruction (Missana MC, 2007). For whole breast reconstruction Serra-Renom used 150, 150 and 100mls in three separate sessions (Serra-Renom et al., 2011).

When injecting into the breast defect there are various methods of injection. Illouz divided the breast into four cosmetic units, systematically treating each breast unit in order to avoid over treatment (IIllouz YG, 2009). As previously stated, injected cords versus large pieces of transferred fat increases graft take (Coleman, 2006). Karacaoglu et al. showed in a rabbit face model that by injecting fat graft into different tissue planes, graft survival is significantly higher when placed in the supramuscular and submuscular layer compared to the subcutaneous layer (Karacaoglu et al., 2005). Translationally this would be similar to injecting into the Pectoralis Major muscle and overlying fascia which is feasible.

1.8 Complications of autologous fat grafting

When compared with other methods of autologous breast reconstruction, the risk associated with autologous fat grafting. Cases of severe complications and death are extremely rare (Surgeons, 2012, Gutowski and Force, 2009). The greatest pitfall of this procedure is the unpredictability of retained graft volumes owing to reabsorption and fat necrosis. Although all forms of breast surgery including reduction mammoplasty, augmentation or flap reconstruction are at risk of fat necrosis (Casey et al., 2013, Chan CW, 2008). The rates from autologous fat grafting seem to be significant enough to require repeat procedures. Earlier reports on fat graft loss due to reabsorption and necrosis were higher with 50-70% after 1 year (I, 1988) Most of the complications of fat necrosis, cyst formation or indurations were found in the first 6 months (IIllouz YG, 2009).

Refinement of techniques has seen improvement with reports is estimated at 30-40% (Delay et al., 2009a). Delay et al. adjusted for this factor by injecting 140% to achieve the expected volume outcomes. Missana suggests that rather than over correct and increasing the risk of cystosteatonecrosis, its preferable to inject over multiple sessions (Missana MC, 2007). Delay suggested that fluctuations in the patient's weight can alter the graft outcome as the adipose tissue maintains characteristics that make it susceptible to weight changes. This has not been demonstrated objectively.

Calcification rates vary with de Blacam noted 3.6% in 111 cases (de Blacam et al., 2011). Missana et al. showed five cases of calcifying liponecrosis in 74 patients (6.7%) which were definitively diagnosed on mammography without further evaluation (Missana MC, 2007). There were no cases of microcalcification

suggestive of malignancy. At follow up MRI (T1weighted) these areas were identified as hypo intense with hyper intense rims.

Cyst formation rates vary with de Blacam noting 1.8% in 111 cases (de Blacam et al., 2011). Wang et al. performed 41 cases of AFT to the breast for cosmetic augmentation. Seven of these patients required removal of 7 nodules owing to patient anxiety. The application of the fat was in only one location, in the retro-mammary area (39). Rietjens reported seven cases in 194 which were all found in radiotherapy patients (Rietjens et al., 2011).

After manipulation of the tissues with cannulae as well as injection of saline there is post-operative swelling (Chan CW, 2008). Two months is recommended before 3D scanning for post op volumes achieved with fat grafting once swelling has subsided.

Cases of minor infection were treated with oral antibiotics. None were severe enough to warrant hospitalization. Rates reported vary from 0.9%-2.7% (Rietjens et al., 2011, de Blacam et al., 2011, IIllouz YG, 2009, Sinna et al., 2010).

The Australian Safety and Efficacy Register of New Interventional Procedures-Surgical (ASERNIPS) systematic review identified two single cases of a single giant liponecrotic pseudocyst and a painful calcified capsule which both required surgical removal (Leopardi D, 2010).

A single case of haematoma and pneumothorax have been recorded (IIllouz YG, 2009, Sinna et al., 2010). A higher number of pneumothoraces were reported recently by Khouri et. al.(Monticciolo et al., 1994)

1.9 Outcomes measures

Oncoplastic surgeons have reported on cosmesis for breast conserving surgery patients. Clough et al. reported prospectively on 101 patients who 82% of success by reporting results as favourable (Petit JY, 2011). This was done using a three-member panel, one surgeon and two non-medical personnel. A grading system of one to five were used (1 = poor and 5 = excellent) with a score of three constituting a favourable result. Since this earlier method of assessing cosmesis Asgeirsson's et al review paper on cosmetic outcomes reported a 18% cosmesis failure rate for breast conserving surgery patients (N=106)(Asgeirsson KS, 2005). Gendy measured cosmesis by a breast retraction assessment (BRA)(Carvajal J., 2008). This tool objectively evaluates the amount of cosmetic retraction of the treated breast in comparison to the untreated breast in patients who receive conservative treatment for breast cancer. To perform measurements a clear acrylic sheet showing 1cm intervals is supported vertically and marked on a grid. Dixon et al. had used a patient questionnaire to assess patient outcomes on 25 patients. Despite no standardised validated method of assessment, both breast retraction and the questionnaire study concluded that immediate reconstruction resulted in better subjective patient satisfaction.

A recent systematic review on breast conserving surgery by Haloua et al. found four papers which reported on cosmesis (Haloua M H, 2013) and these papers differed on manner of assessment and timing of assessment at follow up. Aesthetics varied from either panel member review of the patient or panel review of 2D photographs. Satisfaction was graded by either asking the patient or in the form of a questionnaire. Follow up was six and twelve months or at twelve months post op alone. Quality of life assessment of patients was very limited.

Renewed interest in the concept of autologous fat transfer has produced clinically significant evidence to support patient satisfaction (Pusic, 2010). Outcomes of breast reconstructive surgery have previously been measured by complications and specialist opinion regarding aesthetics achieved. As patients seek reconstruction for psychosocial well-being, we need to spend time considering the patient's perception of outcome. However, the majority of papers have no quantitative or objective measurement. One inquiry was directed at outcomes of surgery on quality of life. Pusic et al. succeeded in constructing an internationally accepted health measurement framework that identifies and quantifies patient opinion on the success of their breast surgery. The BREAST-Q® was developed in 2009 to elicit and quantify patient perception of outcomes post augmentation, reduction, and reconstruction (Pusic, 2003a). These are regarded by quality of life domains in physical well-being, psychosocial well-being and sexual well-being. Satisfaction domains consist of satisfaction with breast, satisfaction with outcome and satisfaction with patient care. Satisfaction with patient care serves as a governance tool to audit and improve services. At a recent International Society for Quality of Life Research conference, Pusic's team presented an extension of the BREAST-Q® to BREAST-Q® BCT module, which will be used specifically in breast conserving surgery patients (Pusic, 2010). In order to examine quality of life in breast conserving surgery patients and ascertain a difference in satisfaction with breast and well-being it would be necessary to assess satisfaction with normal women of comparable age and women who have undergone total mastectomy. Determining the level of dissatisfaction or lack of well-being would help establish whether or not these patients would consider surgical remediation.

Autologous fat transfer for cosmetic outcome and as an adjunct to other reconstructive procedures has developed the interest in its effects on volume, contour, placement and superomedial fullness. Traditional methods of assessment are observation with the naked eye or palpation. More recently digital photography, MRI volume measurement, laser scanners and three-dimensional laser scanners have been employed. Previous methods of objectively and quantifiably measuring the volume of the breast have included water displacement, thermophilic casting and magnetic resonance imaging. Yip J, et al. validated a cyber-ware scanner against the gold standard of water displacement of mastectomy specimens from 30 patients who underwent mastectomy (Yip J.M, 2012). Three-dimensional laser scans using a Cyber ware whole body laser scanner were taken pre and post operatively. Specially designed software was used to calculate the surface area and volume of the breast. The software accounts for the concavity of the chest wall in order to more accurately quantify the breast volume. Thirty-nine breasts were scanned in total and volumes of the scans were compared to the volume of mastectomy specimens. The scanner demonstrated accuracy and reproducibility. After the cost of the purchase of the scanner, individual scans are safe, fast and very economical. Laser scans are likely to become more widely used with the advent of the Vectra 3D scanner, and may replace visual review of 2D photography. Losken et al. showed in 2005 that 3D images preoperatively correlated with mastectomy specimen weight. Kovacs then compared 3D imaging with volumes on MRI (Kovacs L, 2005). The use of 3D scanners is likely to improve operative outcomes as they are used for pre-operative planning and to quantify outcomes.

1.10 Follow up

Illouz suggested in his retrospective series in which 230 patients were followed up for a mean of 11.3 years that yearly MMG or US are advisable yearly (IIllouz YG, 2009). He suggested that any breast lesions, including calcifications, cysts, tumour loco regional occurrence, or primary breast cancer that do not occur 1 year after lipografting is unlikely to be associated with fat grafting. On further review of the literature, there does not seem to be a protocol for timing of imaging pre and post fat transfer for women who have undergone breast cancer surgery and then autologous fat grafting. Imaging was performed 6- 83 months after the procedure in Pierrefeu-Lagrange et al.'s study and Wang et al. used sonography between 2- 17 months (Pierrefeu-Lagrange AC, 2006, Wang et al., 2010). With regard to surveillance, there should be a rigorous protocol for follow up with radiologists who are experienced in breast imaging work, e.g. Breast MRI (Petit et al., 2012, Rietjens et al., 2011). Beck suggested using ultrasound and MRI when indicated to investigate suspicious masses (Beck M, 2011). A breast cancer recurrence imaging protocol does not exist in the literature currently. Most papers describe mammography at either 6 months or 12 month follow up (Rietjens et al., 2011, Petit JY, 2011, Beck M, 2011).

1.11 Future Directions

Stillaert et al. analysed the behaviour of crude fat grafts that were cultured in three dimensional laminin-rich matrix called Matrigel (Corning, 836 North Street, Building 300 Suite 3401 Tewksbury MA 01876, USA) (Stillaert et al., 2010). They demonstrated that human adipose derived stem cells have the capability of multi potential differentiation and have a promising prospect in the field of tissue engineering and regenerative medicine. Stillaert et al. was able to show that harvested fat is a source of multipotent stem cells and fat cells can proliferate in vitro under the right conditions. Freezing of preadipocyte in nitrogen is possible and this is discussed further in chapter 7, whether preadipocytes can be frozen then thawed for use in patients during repeat procedures could be an area of exploration.

As outlined in Browns paper tissue engineering which focuses on adipose derived stem cell (ADSC) therapy, hybrid tissues, scaffolding materials that relate to seeding, viability and cell differentiation patterns is the focus of multiple research groups, which will help understanding of adipose derived stem cells in vivo differentiation (Brown et al., 2010). This will assist advancement of current research into the clinical arena of autologous fat grafting. But currently the role of ADSC's and MSC's in fat grafting for breast cancer patients remains unclear. Further research is likely to be aimed at both the potential or deleterious nature of the introduction of stem cells into the breast post breast cancer surgery. Preadipocytes may have an active role in graft retention rates, oncogenesis or both.

Morrison's chamber model of generating native tissue saw the creation of new adipose tissue around a pedicle flap when an acrylic chamber was placed into the chest (Cronin et al., 2004).The Holy Grail for tissue engineering will be harnessing the differentiation capabilities of adipose derived stem cells. The exciting arena of

stem cell research seems to have boundless applications and potential benefits, but before it can be used possible deleterious effects including oncogenesis need to be explored as well as the applicability, efficacy, cost and patient reported outcomes.

When considering whether or not fat grafting should be undertaken in women who have undergone breast cancer surgery, all clinical studies in the current literature should be systematically reviewed. Is there increased locoregional recurrence when fat grafting is used post breast cancer surgery?

Women requiring the use of AFG for breast reconstruction should be investigated first establishing the quality of life of those women who have not had breast reconstruction, women who have undergone breast conserving surgery and women who have undergone total mastectomy with and without reconstruction. Is there a role for autologous fat grafting for breast reconstruction in these groups of women?

Prospective clinical studies on the efficacy of AFG for whole breast reconstruction, post breast conserving surgery and total mastectomy should be explored using patient reported outcome measures, non-contrast MRI and validated 3D laser scanning. Does fat grafting improve outcomes and is it beneficial for use in breast reconstruction?

Further basic science in vitro laboratory studies investigating the effect of ADSCs on normal breast cells and breast cancer cells will contribute to expanding the field of knowledge regarding autologous fat grafting in breast reconstruction post breast cancer surgery. Do adipose derived stem cells alter proliferation of normal breast cells and breast cancer cell lines?

Chapter 2. Systematic Review of clinical research on fat grafting for autologous breast reconstruction in women who have undergone breast-conserving surgery or total mastectomy

2.1 Introduction

This chapter explores the clinical literature on the use of autologous fat grafting for breast reconstruction in women who have previously undergone breast cancer surgery. A systematic approach was used to identify articles reporting on women who have had either breast-conserving surgery or total mastectomy, with subsequent autologous fat grafting for breast reconstruction. Studies on fat grafting used in combination with autologous flaps, fat grafting pre- and post-radiotherapy, and as an adjunct to implant reconstruction post breast cancer surgery were also reviewed. As fat grafting has only begun to be used in this set of patients in the 21st century, this chapter will outline all aspects of its clinical applications and outcomes and explore whether or not fat grafting causes an increase in locoregional recurrence.

2.2 Background

Women who have undergone breast-conserving surgery lose a segment of breast tissue because the tumour is dissected from the breast with a cuff of normal breast tissue to ensure pathological clearance. If there are multiple tumours within the breast or the breast cancer is aggressive, a total mastectomy is the mainstay of treatment. The options for reconstruction after total mastectomy include autologous flap reconstruction from the abdomen or back. Common abdominal autologous flaps include the deep inferior epigastric perforator (DIEP) and the transverse rectus abdominis myocutaneous (TRAM) flaps. Muscle, adipose tissue, and skin can also be recruited to the chest from the back with the latissimus dorsi flap. Expander and implant reconstruction does not provide additional muscle, fat, or skin to the area, but instead expands the pectoralis major muscle and the overlying skin prior to insertion of a silicone prosthesis. These methods all aim to replace the breast mound so that the breast area approaches its pre-mastectomy appearance. This has been shown to be of great benefit to women, improving psychosocial wellbeing and promoting recovery from the psychological effects of breast cancer (Dean et al., 1983).

The use of fat grafting has multiple applications, from its use for cerebro-spinal fluid leaks in neurosurgery, to reducing burns contracture by softening the scar tissue incurred by severe burns (Klinger et al., 2008). In the last 20 years, fat grafting to the breast has grown in popularity as it is autologous, quicker than the traditional breast reconstruction methods, and can be undertaken as a day surgery procedure with reduced recovery time. Initially used for cosmetic cases only, it has since been used for breast reconstruction. Autologous fat grafting aims to achieve the same

result as traditional methods of reconstruction but due to incidence of fat necrosis and calcification concerns were raised regarding false positive imaging and interference with breast cancer screening. Initially used in cosmetic cases only, it has since been used for breast reconstruction, and although autologous fat grafting seeks to achieve a result comparable to the traditional methods, incidences of fat necrosis and calcification have prompted concerns regarding false positive imaging and interference with breast cancer screening. As improvements in imaging in the 21st century have made it possible to more precisely differentiate fat necrosis from calcifications from breast cancer, this part of the debate has diminished. However, new concerns have since been raised regarding the potential for adipose-derived stem cells to activate quiescent breast cancer cells and promote recurrence (Petit et al., 2012, Vona-Davis and Rose, 2007), but few high quality studies have demonstrated an increase in recurrence rates following fat grafting. Therefore, this issue remains contentious. The American Society of Plastic and Reconstruction Surgeons (ASPS) and Italian Society of Plastic Surgeons have expressed an increased interest in the use of fat grafting for breast reconstruction, but have recommended caution and clinical equipoise. Recently, numerous emerging studies have reported on the use of fat grafting for breast reconstruction in women who have undergone breast-conserving surgery or total mastectomy with minimal adverse outcomes. In fact, recent evidence suggests an improvement in overall results due to enhanced fat harvesting methods and grafting techniques. Moreover, there have been increasing reports of successful outcomes (Delay G., 2009, Delay et al., 2008, Bonomi et al., 2013, Khouri R.K., 2012, Rietjens et al., 2011, Kaoutzanis et al., 2014, Russe et al., 2014, Semprini et al., 2014, Uda et al., 2014, Howes et al., 2014).

The purpose of this review was to systematically identify the manuscripts in the clinical literature on fat grafting in women who have previously undergone breast cancer surgery. The number of women who have had the procedure to date, the number of procedures, the average number of repeat procedures, volumes of fat graft used, any radiological interference with discerning fat necrosis from breast cancer, complications, length of follow up, and breast cancer recurrence were all investigated. Given recent improvements in methods of outcomes assessment such as 3D laser scan technology and validated questionnaires like the BREAST-Q^{TM}, outcome measures were also investigated (Pusic, 2003a). During this review, the strengths and weaknesses of each manuscript were determined in order to establish the overall quality of the literature on this subject.

2.3 Methods

2.3.1 Search strategy

Two investigators (Ben Howes and Chris Xu) used a systematic search strategy to identify studies reporting on fat grafting in women who underwent breast cancer surgery up to December 2016. Without language restriction the databases that were used included MEDLINE, PUBMED, EMBASE, CINAHL, and Clinical Trials Database. The search terms “Autologous fat grafting to the breast”, “Fat grafting outcomes”, “Fat grafting for breast reconstruction” were used. The other medical synonyms were exchanged within the headings were substituted into the initial search phrase and the search was then repeated to identify the maximum number of articles. For example, synonyms for fat grafting included “fat transfer”, “lipofilling,” ”liposculpturing,”and ”structural fat grafting”. Published peer-reviewed articles were included but other sources of identification of literature included book chapters, manually referenced theses, cross-referenced bibliographies, and letters to the editor.

2.3.2 Eligibility criteria

Studies were selected and included in the systematic review through a predetermined protocol:

1) Studies must have involved fat grafting to the breast of patients who had undergone breast-conserving surgery or total mastectomy for breast cancer.

2) Studies must have provided information on at least one of the following relating to outcomes: complications, identification of graft loss, follow-up period, pre- or post-procedure imaging, and techniques used.

3) Studies that reported the use of additives to the fat graft e.g. platelet-rich plasma or cell-assisted fat grafting were excluded. The reason these studies were excluded is because the American Society of Plastic and Reconstructive Surgeons has cautioned that there is less pre-clinical and clinical research into the effects of the additives on oncogenesis. The use of such therapies should be further investigated in pre-clinical research. Hence, only studies in which the patients received their own autologous fat without addition of enhancing substances were included.

4) Case reports were excluded.

2.3.3 Data extraction and quality assessment

The following data was extracted from each study: the first author's last name, publication year, country where the study was conducted, study design, number of subjects, trial period, number of procedures, volume of fat grafted (mean and range), interference with radiology, complications, recurrence of cancer, and any measures of patient satisfaction.

The study quality was assessed using criteria outlined in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement, a validated technique of assessing the quality of observational case-control or case series studies (Ohm, 1827). Individual study methodology was assessed in accordance with the STROBE guidelines for observational studies. The level of evidence of each paper was ranked using the Australian Government National Health & Medical Research Council (NHMRC) levels of evidence and grades for recommendations for developers of guidelines. (Table 1). The STROBE checklist consisted of 21 broad items, but upon review, there was a total of 61 points to be checked in each article. It was therefore established *a priori* that papers would be defined as either excellent, good, average, or poor depending on their overall score out of 61. The overall score was then represented as a percentage. The investigators considered papers to be graded with the following percentage scores: poor <50, average 50-64, good 65-84, and excellent 85-100. Criteria outlined in the STROBE were not applicable to certain papers, e.g. subgroup analysis. In these instances, a "not applicable" (N/A) label was placed and the score was deducted from 61. Incomplete criteria were marked with a half score.

2.3.4 Outcome measures

Patient Cohort: The patient cohort in which fat grafting was used was catalogued and categorized according to the following groups:

i. Breast-conserving surgery followed by autologous fat grafting for defect correction;

ii. Total mastectomy with autologous fat grafting procedures prior to either autologous flap reconstruction or expander implant reconstruction, or as a sole procedure;

iii. Total mastectomy and autologous flap reconstruction (DIEP, Latissimus Dorsi, TRAM, and Free-TRAM reconstructions) with autologous fat grafting used as an adjunct procedure to correct local contour deformity or volume discrepancy.

iv. Total mastectomy and expander/implant reconstruction with autologous fat grafting used as an adjunct procedure.

Exclusion criteria: Manuscripts were reviewed to ascertain the criteria by which certain women were excluded from the clinical trials, and these were documented to determine possible patient selection criteria.

Study Design: The design of the study was catalogued to grade the papers level of evidence based on National Health and Medical Research Council (NHMRC) guidelines (Appendix no. 1).

Duration of Study: This was defined as the period of time in which the study was conducted.

Location and Surgeon: This concerned the country where the study was conducted, whether it was single or multi-centre, and whether there was a single surgeon or multiple surgeons.

Anaesthesia: Whether cases were performed under local anaesthetic, local anaesthetic with sedation, or general anaesthetic was identified.

Technique: Whether authors used the Coleman technique, which has served as the traditional technique, or any deviations from this technique, was determined(Coleman, 2006).

Mean volume injected: This was identified as the amount of fat that was harvested and used per session.

Number of sessions: This was defined as the number of sessions the author used to achieve the aim outlined in their study, and it was counted in each manuscript.

Over-correction: The use of the 30% over-correction outlined by Delay to allow for the resorption losses of the fat graft.

Minor complications: This was defined as conditions that could be monitored on an outpatient basis without inpatient admission for further management.

Major complications: This was defined as conditions requiring an intervention such as ultrasound imaging or IV antibiotics, or conditions that would require further inpatient management, e.g. pneumothorax, stroke from intravascular injection of fat and subsequent emboli, or blindness. Rao and Saadeh's classification system for fat necrosis was used to review the manuscripts that reported on fat necrosis (Howes et al., 2014). Subclinical or level I is defined by the presence of radiological,

intraoperative, or histopathological evidence, while levels II and III include palpable firmness, and level IV is defined as fat necrosis requiring surgical excision.

Resorption rates: Manuscripts were reviewed in terms of their documentation of resorption rates. Ideally, the amount of fat graft loss per session was documented and the method of examination was explained.

Recurrence: This was defined by a recurrence of cancer in the breast that had received fat grafting during a follow-up period.

Follow-up period: This was defined as the period during which patients were followed up to investigate the above stated outcomes, or to complete the process of data collection.

Patient Satisfaction: This included measures to assess patient satisfaction in the form of questionnaires and documentation of comments made by patients during the follow-up period regarding the cosmetic outcomes of the fat grafting procedures.

STROBE Assessment: Each criteria was checked off as per the STROBE checklist (Appendix no. 2).

2.3.5 Bias

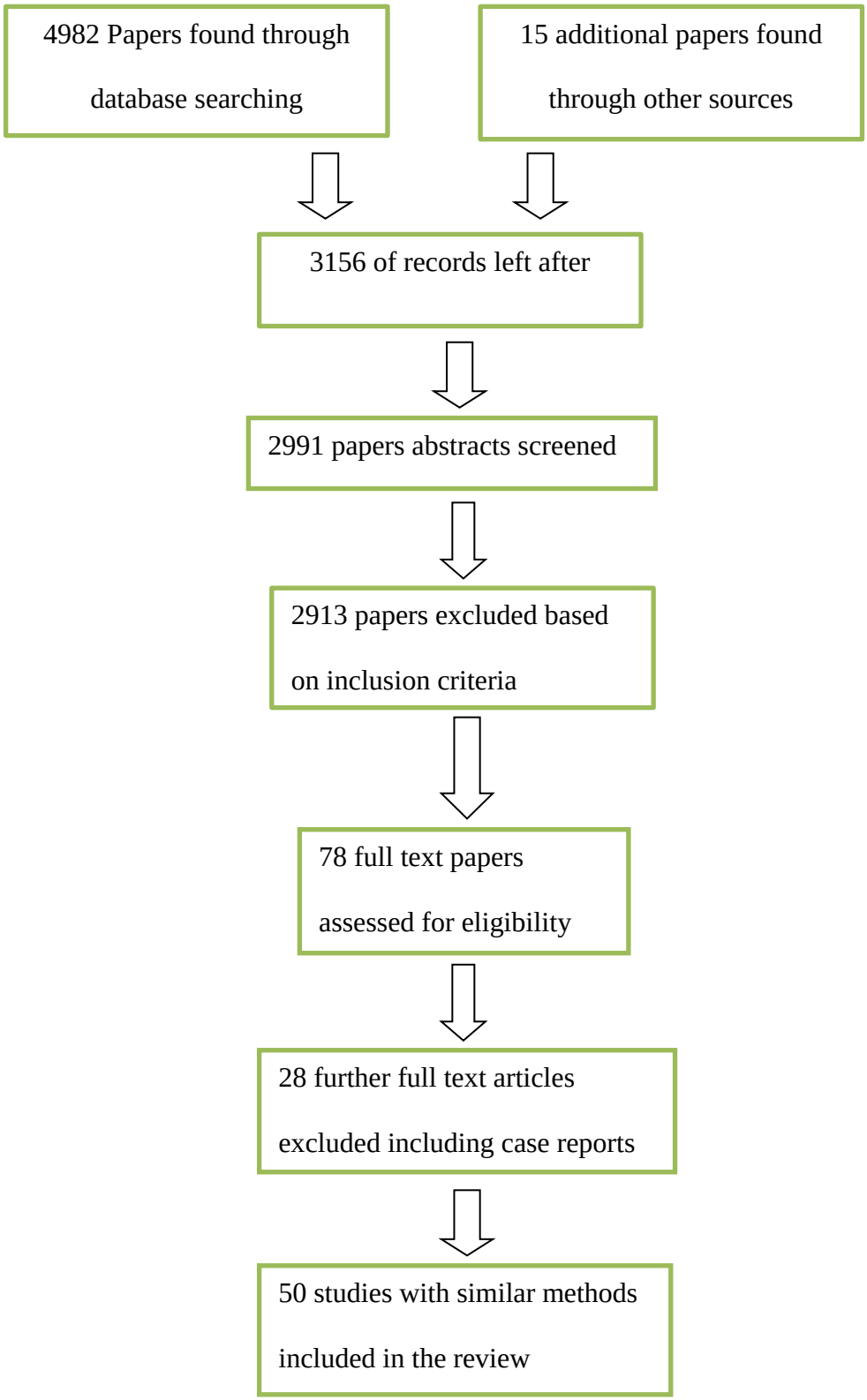
The selection of manuscripts included those written in languages other than English in order to avoid publication bias, and although most abstracts were in English, some were excluded owing to inadequate information to determine eligibility and a lack of a balanced summary of methodology and results. Furthermore, while two papers that satisfied the inclusion criteria were translated into English for data extraction purposes, the standard of translation was not deemed appropriate for STROBE statement scrutiny. This omission could have led to some bias.

2.3.6 Missing data

In instances of missing data relating to the required outcome measures, the corresponding author was contacted by e-mail and requested to provide further information regarding individual patient details specific to the operation that was undertaken, e.g. the volume of fat graft injected or number of repeat procedures. The corresponding authors of papers that neglected to provide detailed information on techniques and complications were also contacted.

2.4 Results

Figure 2-1.Screening and Eligibility Flow Diagram: outlines the finding of the literature search (PRISMA flow diagram).



2.4.1 Manuscripts after review

Thirteen manuscripts were found by reviewing bibliographies for further identification of applicable articles. Book chapters were also reviewed for relevant literature. After screening for the inclusion criteria, 150 papers were excluded due to the following factors: the study was conducted on women who had not undergone breast-conserving surgery or total mastectomy but had other conditions, e.g. Poland's syndrome; the paper consisted of case studies; the information provided was insufficient to qualitatively synthesize the information; or the subject matter was of a strictly cosmetic nature. After duplicate results were eliminated, a total number of 78 articles were found involving fat grafting to the breast in breast conservation and total mastectomy patients (Fig 1.) (Panettiere et al., 2011, Panettiere et al., 2009, Delay G., 2009, Rietjens et al., 2011, Petit et al., 2012, Petit JY, 2011, Petit et al., 1998, Beck M, 2011, Sarfati et al., 2011, Rigotti G., 2005, Kanchwala et al., 2009, Missana MC, 2007, Losken et al., 2011, Sinna et al., 2010, de Blacam et al., 2011, Serra-Renom et al., 2011, IIllouz YG, 2009, Babovic, 2010, Spear SL, 2005, Choi et al., 2013, Perrot et al., 2010, Seth et al., 2012, Parikh et al., 2012, Uda et al., 2014, Ihrai et al., 2013, Bonomi et al., 2013, Hoppe et al., 2013, Salgarello et al., 2011, Gosset J, 2008, Cigna et al., 2012, Ribuffo et al., 2013, Riggio et al., 2013, Semprini et al., 2014, Chaput et al., 2014, Russe et al., 2014, Villani et al., 2010, Alharbi et al., 2013, Mestak and Zimovjanova, 2012, Erol et al., 2010, Serra-Renom et al., 2010, Lancerotto et al., 2013, Maione et al., 2014, Caviggioli et al., 2011). Excluding 12 case studies and a further 16 manuscripts that did not meet inclusion criteria, a total of 50 manuscripts were reviewed by two independent reviewers. One manuscript was designed as a randomized control trial (Lancerotto et al., 2013), and the remainder

were either observational cohort studies, case-control studies, or case series studies, all of which were retrospective in nature. The raw data is outlined in Table 1.

Table 2-1 Data extracted from selected manuscripts

Paper Number	1	2	3	4
Principle author	Rietjens M	Petit JY	Petit JY	Petit JY
Objective	Effect of AFG on complication/imaging/LR	Effect of AFG on complication/imaging/LR	Possibility of increased LR rate in patients with intraepithelial neoplasia	Oncologic outcome between AFG and control groups
Exclusion criteria	Nil	Lack of histology/op data/follow-up < 6months	Metastasis at diagnosis, recurrent BC, other Ca, or breast associated with other Ca	Metastasis at diagnosis, recurrent BC, other Ca, or breast associated with other Ca
Statistical method	Nil	Nil	chi square/univariate cox proportional hazard regression	chi square/univariate cox proportional hazard regression
Design	Prospective cohort	Case Controlled	Case Controlled	Case controlled
Patient makeup	155 BC + 3 non-BC	all BCs (405 invasive ca, 108 Ca in situ)	59: 57 DIN, 2 LIN, 118 control	all BCs
BC surgical procedure	Conservative (62), Mastectomy (93)	Conservative (143), Mastectomy (370)	Conservative 20.3%, Mastectomy 79.7%	Conservative 38.9% (125), Mastectomy 61.1% (196)
No. of patients	158	513	59	321
Mean age (yr.)	48	52.1	49.5	45.5
No. of procedures	194	646	59	321
Duration	36	120	156	121
Location	Single surgeon	Multi centre	IEO data base	IEO data base
Follow up (month)	18.3	19.2	63 vs 66	26
Pre-op evaluation	Clinical, photo, u/s and mammography	Clinical and radiological, otherwise NIIS	NIIS	NIIS

Post-op evaluation	Mammography (MMG), 6 month post-op	Yearly mammography	NIIS	NIIS
Gap (month)	NIIS	39.7	36 vs 56	26, with 6 months of 'washout' in control group
Anaesthetic	Mainly LA and GA	NIIS	NIIS	NIIS
Technique	Coleman's technique	Coleman's technique	Coleman's technique	Coleman's technique
Mean defect size (cc)	19.7	NIIS	NIIS	NIIS
Mean volume of injection (cc)	48	107.3	NIIS	NIIS
Number of injection	>80% of patients had 1 procedure	1.25	NIIS	NIIS
Overcorrection ?	NIIS, but discourage overcorrection	NIIS	NIIS	NIIS
Resorption rate	16.8% needed extra procedures	18.1% needed extra procedures	NIIS	NIIS
Complication	7/194 (fat necrosis, mainly in radiotherapy)	18/646 (fat necrosis, infection, seroma, pneumothorax)	NIIS	NIIS
Interference to radiology	5.2% benign change, 10.6% no data	12/119 in conservatives, with 2 recurrences	NIIS	NIIS
Recurrence	1	5.6% overall	5 yr. cumulative rate 18% vs 3%	8 vs 19, cumulative hazard ratio 1.11 (0.47-2.64, 95% CI)
Satisfaction	NIIS	NIIS	NIIS	NIIS
NHMRC Grade	4	3b	3b	3b

Paper Number	5	6	7	8	9
Principle author	Beck M	Sarfati I	Rigotti G	Sinna R	Panettiere P
Objective	Efficiency of the procedure and impact on imaging and oncological evaluation	AFG in previously Rx prior to implant w/r to: 1) recurrence rate, 2) complication, 3) cosmesis	Estimate LR rate post AFG	Describe preliminary report of patients undergoing lat dorsi and AFG w/o implant	Investigates the usefulness of serial fat grafting in irradiated prosthetic breast reconstructions
Exclusion criteria	Nil	Nil	Conservative pts were excluded	NIIS	NIIS
Statistical method	Nil	Nil	Dependent group analysis	Nil	Student t and Wilcoxon
Design	Prospective study	Prospective cohort	Cohort study	Case series	Clinical Trials
Patient makeup	Consecutive BC pts +/- post-op radio +/- chemo	Consecutive BC pts who had radiotherapy +/- chemo +/- hormonal therapy	BC pts	BC pts	BC pts
BC surgical procedure	Lumpectomy	Mastectomy	Radical mastectomy	Lat dorsi recon	BC
Number of patients	10	28	137	200	20
Mean age (yr.)	49	45	46.5	48.7	49.1
Number of procedures	10	28	137.00	244	20
Duration	48	24	60	48	24
Location	Single surgeon	Single surgeon	Single surgeon	Single surgeon	NIIS
Follow up (month)	36	17	91.2	14.5	17.6
Pre-op evaluation	Mammogram and CT	Patient and surgeon	NIIS	3D scan and photography	LENT-SOMA

Paper Number	5	6	7	8	9
Principle author	Beck M	Sarfati I	Rigotti G	Sinna R	Panettiere P
Post-op evaluation	Patients evaluation, CT & 3D scan at 36 month. Additional MRI and u/s if indicated	Patient and surgeon, 4.6 months between last AFG and prosthesis	NIIS	Surgeon and Patients report	LENT-SOMA 3 months post last AFG
Gap (month)	NIIS	6	23 (same as control)	11.7 post recon	NIIS
Anaesthetic	80% LA, 20% GA	NIIS	LA with sedation	mainly GA	NIIS
Technique	Coleman's technique	Coleman's technique	Rigotti's modificatio	NIIS, centrifuge	No centrifugation, Coleman
Mean defect size (cc)	NIIS	NIIS	NIIS	NIIS	NIIS
Mean volume of injection (cc)	67.5	115	NIIS	176	24.5
Number of injection	1	1~3, delay of 3.3 months in between sessions	2-4 sessions	1-3 sessions, 58/244 repeat procedures	3.4 average (with 2.5 months in between)
Overcorrection?	Usual clinical overcorrection 30-50%	NIIS	NIIS	Yes	NIIS
Resorption rate	8.9% after 1/12; 11% after 3/12 53% after 3yrs	NIIS	NIIS	~30%	NIIS
Complications	NIIS	4 seromas (1 prosthesis exposure)	NIIS	8/200 (2 local infection, 1 pneumothorax, 5 fat necrosis)	AFG salvaged 4 potential implant exposures
Interference to radiology	Discernible fat necrosis	NIIS	NIIs	NIIS	NIIS
Recurrence	NIIS	NIIS	cumulative 6.5%	NIIS	NIIS
Satisfaction	NIIS	80% good to very good judged by patients/panel	NIIS	NIIS	aesthetic outcome 4.3 vs 3.1
NHMRC Grade	4	4	4	4	4

Paper Number	10	11	12	13
Principle author	Missana MC	Seth AK	Salgarello M	de Blacam C
Objective	To study sequelae of AFG	Evaluate implant recon outcomes in women +/- AFG	AFG post radiotherapy prior to implant recon	Review the author's early experience of AFG after post mastectomy breast recon
Exclusion criteria	NIIS	Lack of complete pathology	strict oncological follow up and free of disease;, 6 month GAP post radio	Inadequate follow up time (<3month) or lost to follow
Statistical method	Nil	Fisher's exact test, t test	Nil	Fisher's exact or chi-square and t test
Design	Retrospective cohort study	Case Controlled	Retrospective cohort	Case series
Patient makeup	BC pts	BC and non-BC pts (negative pathology)	BC pts	
BC surgical procedure	Combination of conservative/prosthesis/Lat dorsi+/-prosthesis and TRAM	Mx immediate tissue expander recon, then 2nd stage implant	5 conservative+radio; 11 mastectomy+radio	Mastectomy + recon, with 19/68 breasts had irradiation
Number of patients	69	68	16	49
Mean age (yr.)	51	48	41	47.4
Number of procedures	74	99	51	111
Duration	48	120	36	24
Location	NIIS	Single centre	NIIS	Single centre (two surgeons)
Follow up (month)	11.7	42.1 vs 43.6 (24.8 post AFG)	15 post definitive implant	mean follow up was 2.4yrs
Pre-op evaluation	MRI prior; 2 surgeons	NIIS	LENT-SOMA prior to implant	Photograph

Paper Number	10	11	12	13
Principle author	Missana MC	Seth AK	Salgarello M	de Blacam C
Post-op evaluation	MRI at 3 months; 2 surgeons	NIIS	BREAST-Q score post implant	photograph, >3months post AFG with 2 plastic surgeons review
Gap (month)	NIIS	18.3	3 after mastectomy in previous radio pts, or 6 post radio	minimum 2 months, average 12.4 months
Anaesthetic	GA	NIIS	NIIS	GA
Technique	Coleman's	Coleman's	NIIS, centrifuge	NIIS, centrifuge
Mean defect size (cc)	NIIS	NIIS	NIIS	NIIS
Mean volume of injection (cc)	75-107	20-50 per breast	88.6 in conservative, 102.8 in mastectomy	67 per treatment, 107 overall
Number of injection	1.04-1.67	1-4 sessions	2-3 sessions	1-4 sessions, 51.5% breasts required more than 1 injection
Overcorrection?	No	NIIS	Nil	NIIS
Resorption rate	14.86% needed an extra procedure	NIIS	NIIS	NIIS
Complications	5/74, fat necrosis	1 in AFG, fat necrosis	1 Baker-1 capsular contracture	7/111, 6.3%
Interference to radiology	Nil	Nil	NIIS	NIIS
Recurrence	NIIS	17	NIIS	NIIS
Satisfaction	86.5% good-v good judged by panel	NIIS	97.3% satisfaction	NIIS
NHMRC Grade	4	3b	4	4

Paper Number	14	15	16	17	18
Principle author	Delay E	Serra-Renom J	Spear S	Bonomi R	Ihrai T
Objective	Retrospective review of technique and results in 880 AFG procedures	Usefulness implant, and AFG in patients who had Rx	Review the safety of AFG	Effect of AFG in patients who had lat dorsi +/- implant	Study cancer recurrence and post-AFG imaging
Exclusion criteria	NIIS	No 'radio dermatitis', GAP>12 months	NIIS	Inclusion: mastectomy and recon patient	Follow up less than 12 month in given years
Statistical method	Nil	Nil	Nil	Nil	Nil
Design	Prospective case series	Case series	Case series	Retrospective cohort	Case series
Patient makeup	Mixture of cohorts: breast recon/congenital deformities/aesthetic/correction for previous defect	BC pts with radiotherapy	Mixed cohort	BC pts	BC pts
BC surgical procedure	Mixed	Mastectomy	Not all BC pts	Mastectomy + recon	Mastectomy and conservative, with implant/lat dorsi recon
No. of patients	880	65	37	31	64
Mean age (yr.)	NIIS	NIIS	NIIS	55	
No. of procedures	880	137	47	44	100
Duration	120	36	120	36	60
Location	Single surgeon	NIIS	Single surgeon	Single surgeon	Single surgeon
Follow up (month)	At least 12 months, but already 10yrs since 1st pt.	12 months	*11-15 months	21 months	46.4 months
Pre-op evaluation	Evaluation	NIIS	2D photograph	2D photograph	NIIS

Paper Number	14	15	16	17	18
Principle author	Delay E	Serra-Renom J	Spear S	Bonomi R	Ihrai T
Post-op evaluation	NIIS	Patient/nurse/surgeon and Baker's classification	2D photograph	OPD for clinical exam and Picker questions	NIIS
Gap (month)	NIIS	Recon performed >1yr post mastectomy	NIIS	NIIS	78.8
Anaesthetic	GA in majority	NIIS	NIIS	GA	NIIS
Technique	Coleman's technique	Coleman's technique	NIIS	Coleman's technique	Coleman's technique
Mean defect size (cc)	NIIS	NIIS	NIIS	NIIS	NIIS
Mean volume of injection (cc)	Lat dorsi, 200mL; implant, 50-100mL	Stage 1: 150mL+/-25, stage 2: 150mL+/-30mL	116mL	247mL	1st AFG 38mL, 2nd AFG 60mL
Number of injection	NIIS	1 injection per 1st/2nd stage, patient may need 3rd or 4th injection	Most patient had 1 session, 3 pts had 2 and 1 had 3	1-3, mainly 1 session	1.57, ranging 1-5 sessions
Overcorrection?	Yes, 140%	NIIS	NIIS	Yes, 130%	NIIS
Resorption rate	30-40% resorption rate at 6/12 mark	NIIS	NIIS	NIIS	NIIS
Complications	6/880	No complications	4/47, 8.5%: 1 cellulitis and 3 fat necrosis	recurrence, 2 fat necrosis and 1 oil cyst	1/64: infection at harvesting area
Interference to radiology	Nil	NIIS	NIIS	NIIS	1 anomaly, scar tissue biopsy
Recurrence	NIIS	NIIS	NIIS	1	2
Satisfaction	NIIS	Satisfaction rating was 4/5, Baker's contracture </=1	Aesthetic improvement: 10/43 (21%); minimal to moderate 30/43 (64%);	NIIS	NIIS
NHMRC Grade	4	4	4	4	4

Paper Number	19	20	21	22	23
Principle author	Kanchwala S	Uda H	Losken A	Costantini M	Parikh R
Objective	Oncogenesis review of recurrence post AFG	Case series of mastectomy pts managed by BRAVA+AFG	Review experience with fat grafting for the correction of acquired breast deformities	To describe radiological appearance of normal and pathological finding resulting from AFGs	Develop imaging classification to differentiate fat necrosis from recurrent cancer
Exclusion criteria	No cosmesis/lumpectomy filling	If local or remote recurrence	NIIS	NIIS	Not experiencing palpable mass, lack of imaging or having less than 1 yr. follow up.
Statistical method	Nil	Nil	X2 cross tabulation test	two tails t test	Nil
Design	Case series	Case series	Retrospective review	Retrospective review	Retrospective review
Patient makeup	Most BC pts, no clear indication	6 irradiated total mastectomy; 8 non-irradiated conservative lumpectomy	BC pts +/- radiation	BC pts (92%) and Poland (1) and asymmetry (1) +/- radiotherapy	BC pts and risk-reduction pts + recon
BC surgical procedure	TRAM/Expander/lat dorsi+exp/3 unknown	Mastectomy only and lumpectomy+radiotherapy	TRAM 55; Implant 20, Lat dorsi 20; BCT 12	Mixed	Bilateral mastectomy (28/37), Unilateral mastectomy (9/37)
Number of patients	110	14	107	22	37
Mean age (yr.)	49.3	50	52	50.8	53
Number of procedures	110	32	142	46	82
Duration		72	168	24	60
Location	Single surgeon	Single surgeon	Single surgeon	Single surgeon	Single surgeon

Follow up (month)	21 months	At least 6/12	at least 6/12, average 8/12	12	6.5
Pre-op evaluation	Patient and surgeon evaluation	2D photograph	NIIS	NIIS	u/s

Paper Number	19	20	21	22	23
Principle author	Kanchwala S	Uda H	Losken A	Costantini M	Parikh R
Post-op evaluation	Patient and surgeon evaluation	Photograph+3 plastic surgeons, 6/12 MRI	Patient satisfaction	Mammogram, u/s, MRI	u/s +/- biopsy
Gap (month)	At least 3 months	Average 22/12 post BC operation	NIIS	NIIS	NIIS
Anaesthetic	NIIS	NIIS	NIIS	NIIS	GA
Technique	Coleman	Coleman+ centrifuge	Lipovage/Telfa pads, sediment and centrifuge	Coleman's technique + centrifuge	NIIS
Mean defect size (cc)	NIIS	NIIS	NIIS	NIIS	NIIS
Mean volume of injection (cc)	31 mL	256 mL	40 mL initial, 44.3 mL subsequent	114.8 mL	42.77
Number of injection	61/110 pts had multiple injections	1-4 sessions	80/107 had 1 session	2.2 per patient	2.22 per patient
Overcorrection?	up to 10%	NIIS	NIIS	NIIS	Nil
Resorption rate	NIIS	NIIS	NIIS	NIIS	NIIS
Complications	NIIS	2/14, fat lysis and cellulitis	12/107, 11%	45 oil cyst, 7 fat necrosis	66 lesions in 37 patients, 22/66 were biopsied
Interference to radiology	Nil	NIIS	NIIS	No interference	NIIS
Recurrence	NIIS	NIIS	NIIS	1	1
Satisfaction	Good to excellent result in 855 of all pts	NIIS	Response rate 17/23, 14/17 had improvement.	NIIS	NIIS
NHMRC Grade	4	4	4	4	4

Paper Number	24	25	26	27	28
Principle author	Cigna E	Illouz	Gossette J	Russe	Hoppe
Objective	Review previous fat grafting after breast reconstruction with prosthesis	Review of single surgeon cases	Investigate the radiological impact of AFG	Investigate AFG for recon	Total mastectomy and fat grafting follow up protocol
Exclusion criteria	Post-op radiotherapy	BI-RADs>2	Pre-intervention imaging - no local recurrence	Not mentioned	NIIS
Statistical method	Student t test	Nil	NIIS	No statistics mentioned	NIIS
Design	Retrospective cohort study	Retrospective cohort	Retrospective cohort study	Retrospective analysis of surgical and outpatient reports	Retrospective case series
Patient makeup	BC pts	Mastectomy	BCS patients	BCS patients	Total mastectomy
BC surgical procedure	Nipple/skin and skin reducing mastectomy	Mastectomy	BCS only	Fat grafting	Total mastectomy
number of patients	20	381	21	187	28
mean age (yr.)	65	45.6	50.7	46	52.4
number of procedures	20	381	54	298	135
Duration	36	252	60	64	21
Location	Single surgeon	Single surgeon	Single surgeon	Single surgeon	Multi-centre
Follow up (month)	12	11.3 years	MMG, US, MRI at 1 year post AFG	Not mentioned	30
Pre-op evaluation	VAS score, patient and surgeon	Nil	Mammography, US, MRI	Not mentioned	us, mmg

Paper Number	24	25	26	27	28
Principle author	Cigna E	Illouz	Gossette J	Russe	Hoppe
Post-op evaluation	VAS score, patient and surgeon	MMG or U/S 6mnths-1yr with BI-RADS	1 year: MMG, US, MRI	NIIS	US, MMG, , patient questionnaire (10-Likert scale), digital photographs.
Gap (month)	NIIS	NIIS	NIIS	Not mentioned	NIIS
Anaesthetic	GA	GA	NIIS	General anaesthetic	NIIS
Technique	Coleman's +centrifuge	Authors own	NIIS	Lipivage™-System.	BEAULI™ method
Mean defect size (cc)	NIIS	NIIS	NIIS	NIIS	NIIS
Mean volume of injection (cc)	NIIS	145 (total volume over 3 sessions 500mls)	166	90 ml	159
Number of injection	NIIS	Average 3 per patient	1.3 per patient	1 (42%), 2 (31%), >2 (27%)	NIIS
Overcorrection?	20-25%	No	NIIS	Not mentioned	NIIS
Resorption rate	NIIS	NIIS	NIIS	Say it is hard to measure	NIIS
Complications	1/20, fat necrosis	5 infections, haematoma	micro calcifications (19%), cysts on 57% U/S, whereas 47% on MRI	3.9% (contour deformation, infections)	Fat necrosis (2.59%), infection (0.74%), haematoma (0.74%) and granuloma (0.74%)
Interference to radiology	NIIS	Finding descriptive	Similar to other procedure, clearly benign	Not mentioned	Minimal, only granuloma (0.74%)
Recurrence	NIIS	NIIS	1	NIIS	NIIS
Satisfaction	VATS 4.9-7.1, 6/12 later	NIIS	NIIS	NIIS	Patient satisfaction high (96%), good aesthetic results (68%)
NHMRC Grade	4	4	4	4	4

Paper Number	29	30	31	32	33	34	35
Principle author	Semprini	Ribuffo	Riggio	Longo	Gale	Amar	Pierrefeu-Lagrange
Objective	Review oncological recurrence after AFG	If AFG reduces complications in irradiated breasts with expanders	Investigate loco regional recurrence after AFG	Define a systematic approach with AFG	Assess oncological risk	Evaluate AFG post BCS	Evaluate imaging post AFG
Exclusion criteria	NIIS	Nipple sparing and skin sparing mastectomy	NIIS	T2DM, smoking, PVD, local or distal metastasis	Benign disease, , recurrence prior to AFG, failure to identify case match	NIIS	NIIS
Statistical method	NIIS	Fishers exact test	Not specified	Student t test, Kruskal-wallis test	Chi-squared test	NIIS	NIIS
Design	Retrospective cohort study	Retrospective case control study	Prospective cohort study	Prospective study	Case-controlled	Prospective	Retrospective cohort
Patient makeup	BCS	Two stage expander implant patients post radiotherapy	Flaps, implants	NSM with or without radiation	Mastectomy, BCS, Control	BCS	Lat Dorsi + AFG
BC surgical procedure	BCS	Modified radical mastectomy	Phylloid and sarcoma	NSM	Mastectomy & BCS	BCS	Total Mastectomy
Number of patients	151	16	60	21	211	15	30
Mean age (yr.)	NIIS	49.5	49	36.6	42	NIIS	51
Number of procedures	151	23	82	11	42	15	34
Duration	24	48	84	60	72	1	72
Location	Single surgeon	Single surgeon	Single surgeon	multiple surgeon	Single surgeon	Single surgeon	Single surgeon
Follow up (month)	45	12	120	20.6 vs 31.7	140	9 months	NIIS
Pre-op evaluation	NIIS	None specified	NIIS	Digital photography with grading	NIIS	MMG, photographs, patient satisfaction	MMG, US, MRI

Paper Number	29	30	31	32	33	34	35
Principle author	Semprini	Ribuffo	Riggio	Longo	Gale	Amar	Pierrefeu-Lagrange
Post-op evaluation	NIIS	US	US	Digital photo grading scale		MMG, patient satisfaction, panel, 3D MRI	MMG, US, MRI
Gap (month)	NIIS	3.5	55	Less than 6/12 vs >6/12	56	NIIS	NIIS
Anaesthetic	NIIS	GA, LA and sedation	LA	NIIS	NIIS	Local and GA	NIIS
Technique	NIIS	Coleman's technique	Coleman's technique	Dry technique, centrifuge	Coleman's technique	Coleman's technique	NIIS
Mean defect size (cc)	NIIS	NIIS	NIIS	NIIS	NIIS	NIIS	NIIS
Mean volume of injection (cc)	NIIS	109	47	137 vs 109 per injection per breast	NIIS	63	165
Number of injection	NIIS	23	82	33 vs 55	4.14 vs 4.17	NIIS	NIIS
Overcorrection?	NIIS	No	NIIS	130% rule	NIIS	NIIS	NIIS
Resorption rate	NIIS	NIIS	NIIS	NIIS	NIIS	48%	NIIS
Complications	NIIS	No complications	2 recurrences	Nil	NIIS	1 infection	14 fat necrosis
Interference to radiology	NIIS	NIIS	None	US 6/12 and MMG 12/12,	NIIS	Nil	For biopsy in some cases
Recurrence	NIIS	NIIS	2	NIIS	4	NIIS	NIIS
Satisfaction	NIIS	NIIS	NIIS	Subscale analysis higher in Group A	NIIS	NIIS	NIIS
NHMRC Grade	4	4	4	4	3b	4	4

Paper Number	36	37	38	39	40
Principle author	Masia	Doren	Biazus	Khouri	Rigotti
Objective	Compare recurrence post AFG after implant recon	Examine fat necrosis after AFG	Viability of AFG following BCS	Review BRAVA and AFG	Treating radiation damage with AFG
Exclusion criteria	Recurrence, conservative management, did not attend follow up	Lumpectomy and AFG	Inclusion: invasive breast ca (stage 1 and 2) who had BST	Smoking, prolonged bleeding, previous liposuction, radiation	No radiation
Statistical method	T-test, Chi squared test	Wilcoxon rank sum test and chi-square test	NIIS	NIIS	T-test
Design	Retrospective Case Controlled	Retrospective review	Prospective study	Retrospective	Prospective
Patient makeup	Flap recon vs AFG	Mastectomy patient (with or without BC) +/- chemo/Rtx	Breast CA with RTx +/- chemo	BCS, immediate and delayed recon	Mastectomy
BC surgical procedure	Total Mastectomy	Mastectomy	Breast conservative therapy	BCS, mastectomy	Mastectomy
Number of patients	100	278	20	488	20
Mean age (yr.)	49	51	55.4	NIIS	51
Number of procedures	107	278	20	1877	45
Duration	264	60	12	84	
Location	Single surgeon	Single surgeon	Single surgeon	Multicentre	Single surgeon
Follow up (month)	60	28	29	84	31
Pre-op evaluation	NIIS	Exclusion/inclusion criteria	2D photography	Unclear	Lent soma grading

Paper Number	36	37	38	39	40
Principle author	Masia	Doren	Biazus	Khouri	Rigotti
Post-op evaluation	MMG and clinical exam	Breast and plastic follow up, with u/s +/- biopsy. Pt and plastic surgeon self reporting system	Clinical exam, photography, mammography (6/12)	Clinical follow up	Lent soma grading
Gap (month)	NIIS	16.7	Immediate	NIIS	None
Anaesthetic	NIIS	NIIS	NIIS	NIIS	General
Technique	NIIS	Coleman's technique and gravity	Coleman's technique and centrifuge	Khouri technique	Coleman's technique
Mean defect size (cc)	NIIS	NIIS	56	NIIS	NIIS
Mean volume of injection (cc)	99.5	50	121	225	70
Number of injection	210	NIIS	1	1877	45
Overcorrection?	NIIS	NIIS	Overcorrection, predicting 30-50% resorption	NIIS	NIIS
Resorption rate	NIIS	NIIS	NIIS	NIIS	NIIS
Complications	NIIS	Fat necrosis and oil cyst in 23%, 6% required needle or excisional biopsy,	4 seroma, 1 fat necrosis, 1 wound infection	5 pneumothoraces, 20 ulcerative infections, 12% benign palpable lesions (36% in irradiated breasts)	NIIS
Interference to radiology	NIIS	NIIS	1 patient has BI-RADS LvL3, biopsy confirmed fat necrosis	None	NIIS
Recurrence	3	0	0	3	0
Satisfaction	NIIS	31% v satisfied; 36% mostly satisfied;	Patient score 9.45; doctor 8.78	NIIS	NIIS
NHMRC Grade	4	4	4	4	4

Paper Number	41	42	43	44	45
Principle author	Coleman	Bezerra	Brenelli	Choi	Kautzonis
Objective	Evaluation of AFG	Retrospective review of AFG	Oncological Safety of AFG	Graft retention rates	Complication rates including local recurrence
Exclusion criteria	None specified	None specified	NIIS	None specified	None specified
Statistical method	None specified	None specified	NIIS	None specified	Descriptive statistics
Design	Retrospective	Retrospective	Prospective	Prospective	Retrospective
Patient makeup	Micromastia, tuberous breasts, post mastectomy, Poland's	Total mastectomy and BCS	BCS	Lumpectomy, secondary to flaps, revision of implants	Unilateral and bilateral mastectomy
BC surgical procedure	Total Mastectomy	Total and BCS	BCS	not specified	Total mastectomy
Number of patients	2	112	59	81	104
Mean age (yr.)	45		50	49.6	48
Number of procedures	3	112	75	123	104
Duration	60	96	36	None specified	60
Location	Single surgeon	Single surgeon	Single surgeon	Single surgeon	Single surgeon
Follow up (month)	12	9	34	140	57
Pre-op	MMG	NIIS	MMG and US	3D image	NIIS

Paper Number	41	42	43	44	45
Principle author	Coleman	Bezerra	Brenelli	Choi	Kautzonis
Post-op evaluation	MMG	NIIS	MMG and US	3D image	Lesion biopsy
Gap (month)	NIIS	NIIS	76.6	NIIS	NIIS
Anaesthetic	GA, LA/sedation, epidural + sedation	LA	LA or GA	Not specified	NIIS
Technique	Coleman's technique	Authors own	Coleman's technique	Modified Coleman's	Coleman's technique
Mean defect size (cc)	NIIS	NIIS	NIIS	NIIS	NIIS
Mean volume of injection (cc)	124	100	52	151 (111-216)	95
Number of injection	3	NIIS	NIIS	1	NIIS
Overcorrection?	NIIS	NIIS	NIIS	No	NIIS
Resorption rate	NIIS	NIIS	NIIS	40-50%	NIIS
Complications	1 nodule	22 palpable nodules	20%	NIIS	36.1% benign lesions, no recurrence
Interference to radiology	Nil	NIIS	8% required biopsy	NIIS	3.7% suspicious lesion were benign
Recurrence	0	NIIS	4%	NIIS	none
Satisfaction	NIIS	NIIS	NIIS	NIIS	NIIS
NHMRC Grade	4	4	4	4	4

Paper Number	46	47	48	49	50
Principle author	Juhl	Caviggioli	Maione	Weichman	Zhu
Objective	Effect of fat grafting to alleviate post mastectomy pain	AFG to relieve PMPS	Effect of AFG on pain post mastectomy	Observe difference between micro flap and AFG	Enhancing volume of LD with AFG
Exclusion criteria	None specified	None specified	Chemo, post op complications, local recurrence	Non flap recon	NIIS
Statistical method	ANOVA	Wilcoxon rank test	Wilcoxon rank test	Wilcoxon rank test	NIIS
Design	Randomised	Prospective	Prospective	Retrospective	Retrospective
Patient makeup	Mastectomy vs control	Mastectomy	Mastectomy	Flap recon	Flap recon
BC surgical procedure	Unilateral Total Mastectomy	Total mastectomy	BCS	Mastectomy	Total mastectomy
Number of patients	8	72	57	100	10
Mean age (yr.)	60	NIIS	51	NIIS	55
Number of procedures	8	63	57	100	14
Duration	30	30	12	48	24
Location	Single surgeon	Single surgeon	Single surgeon	Single surgeon	Single surgeon
Follow up (month)	6	13	10	NIIS	NIIS
Pre-op evaluation	VAS, Neuropathic pain inventory, scar assessment	Clinical, VAS	Clinical, VAS	BMI	NIIS

Paper Number	46	47	48	49	50
Principle author	Juhl	Caviggioli	Maione	Weichman	Zhu
Post-op evaluation	VAS, Neuropathic pain inventory, scar assessment	Clinical, VAS	Clinical, VAS	BMI	NIIS
Gap (month)	19	NIIS	NIIS	NIIS	NIIS
Anaesthetic	GA	NIIS	NIIS	GA	GA
Technique	Coleman's technique	Coleman's technique	Coleman's technique	Coleman's technique	Revolve technique
Mean defect size (cc)	NIIS	NIIS	NIIS	NIIS	NIIS
Mean volume of injection (cc)	71	55	39	147.8	176
Number of injection	1	NIIS	NIIS	112	NIIS
Overcorrection?	no	NIIS	NIIS	NIIS	NIIS
Resorption rate	NIIS	NIIS	NIIS	NIIS	NIIS
Complications	None	NIIS	NIIS	Related to flap rather than AFG	NIIS
Interference to radiology	NIIS	NIIS	NIIS	NIIS	NIIS
Recurrence	NIIS	NIIS	NIIS	NIIS	NIIS
Satisfaction	NIIS	NIIS	NIIS	NIIS	NIIS
NHMRC Grade	2	4	4	4	4

2.4.2 NHMRC and STROBE results

NHMRC guidelines for assessing the level of evidence were used for each manuscript. The NHMRC evidence grades determined that the highest paper in this review met the criteria for level 11 evidence, despite the paper's very small study size (Lancerotto et al., 2013). The vast majority of papers were level IV evidence. Using the STROBE checklist, one paper included the majority of the STROBE criteria and was ranked "excellent", and the same author produced another "good" paper. A total of twelve studies were ranked "good". Twenty-one papers received "average" scores, as they fulfilled approximately 57% (mean) of the STROBE criteria. Four papers were not balanced in terms of the reporting of methods or results and were ranked "poor". The majority of papers were retrospective, with only sixteen prospective papers in the review.

Table 2-2 Strobe Assessment

		Choi M	Losken A	Costantini M	Parikh R	Uda H	de Blacam C	Sinna R	Serra-Renom
STROBE Criteria	TITLE AND ABSTRACT	Prospective	Retrospective	Retrospective	Retrospective	Prospective	Retrospective	Retrospective	Retrospective
1.1	Indicate Design	P	P	P	P	P	P	P	P
1.2	Informative and balanced on method	P	P	P	P	P	P	P	P
1.3	Informative and balanced on results	P	P	P	P	P	P	P	P
2.1	Background: Explain background	P	P	P	P	P	P	P	P
2.2	Rationale	P	P	P	P	P	P	P	P
3	Objective: State objective	P	P	P	P	P	P	P	P
4	Key element of the study design	P	P	P	P	P	P	P	P
5.1	Setting: Describe setting	P	P	P	P	P	P	P	P
5.2	Location	P	P	P	P	P	P	P	A
5.3	Date	A	A	A	A	P	P	P	P
5.4	Recruitment Period	A	P	P	P	P	P	P	P

5.5	Exposure	P	i	P	P	P	P	P	P
5.6	Follow up	P	P	P	P	P	P	P	P
5.7	Data collection	P	A	P	P	P	P	P	P
6.1	Participants: Describe criteria	i	i	A	A	P	P	P	P
6.2	Source	P	P	P	P	P	P	P	P
6.3	Method of selection	i	P	A	P	P	P	P	P
6.4	Method of follow up	P	P	P	P	P	P	P	P
6.5	Matching criteria	na	na	na	na	na	na	na	na
7.1	Variables: Define outcomes	P	A	A	P	P	P	P	P
7.2	Exposure	P	A	P	P	P	P	P	P
7.3	Predictors	A	A	A	A	A	A	A	A
7.4	Potential confounders	A	A	A	A	A	A	A	A
7.5	Effect Modifiers	A	A	A	A	A	A	A	A
8.1	For variable of interest give data source	i	A	A	A	P	P	P	

STROBE Criteria		Choi M	Losken A	Costantini M	Parikh R	Uda H	de Blacam C	Sinna R	Serra-Renom

8.2	Details of measurements	P	A	A	A	A	A	A	A
8.3	Comparability method of groups	P	A	A	A	A	A	A	A
9	Bias: Effort to address potential bias	A	A	A	A	A	A	A	A
10	Study size: Describe how study size is arrived	A	A	A	P	P	P	P	P
11	Describe how variable were handled in analysis	na	na	na	na	A	A	A	A
12.1	Describe all statistical methods	na	P	P	na	P	P	P	na
12.2	Describe any methods used to examine subgroups and interactions	na	A	A	na	na	na	A	na
12.3	Explain how to address missing data	A	na	na	na	na	na	na	na
12.4	Cohort study (how to address loss of follow up);	A	P	na	na	na	na	na	na
12.5	Describe any sensitive analysis	na	na	na	na	na	na	na	na
13.1	Examine and number of eligible participants	P	P	P	P	P	P	P	P
13.2	Completed participation	P	P	P	P	P	P	P	P
13.3	Follow up	P	P	P	P	P	P	P	P
13.4	Analysis	P	P	P	P	P	P	P	P
13.5	Give reasons for non-participants	na	na	na	P	na	na	na	na
13.6	Consider use of flow diagram	na	na	na	na	na	na	na	na
14.1	Characteristics of study participants	i	P	P	P	i	P	i	A

14.2	Information on exposure to potential confounders	A	P	A	A	A	A	i	A
14.3	Missing data for each variable of interest	na	na	na	na	na	na	na	na
14.4	Cohort study: summarise follow up time	P	P	na	na	na	na	na	na
15	Cohort study (report numbers of outcome event)	P	P	na	na	na	na	na	na
16.1	Give unadjusted estimates	A	P	P	P	P	P	P	P
16.2	If applicable confounder adjusted estimates and their precision	na	P	na	na	A	A	A	A
16.3	Report category boundaries	A	na	na	na	i	na	na	na
16.4	If applicable, consider translating relative risk to absolute risk	na	na	na	na	na	na	na	na

STROBE Criteria		Choi M	Losken A	Costantini M	Parikh R	Uda H	de Blacam C	Sinna R	Serra-Renom J
17.1	Other analysis: Report analyses for subgroups	P	P	P	na	i	i	A	na
17.2	Interaction	P	P	P	na	A	A	A	A
17.3	Sensitivity analysis	P	na	P	na	A	A	A	A
18	Summarize key results with reference to study objectives	P	P	P	P	P	P	P	P
19.1	Limitation: Discuss limitation of the study	A	A	P	A	A	P	A	A
19.2	Bias	A	A	A	A	A	A	A	A
20.1	Cautious overall interpretation of results	P	i	P	P	P	P	P	P

20.2	Limitation	A	A	A	A	A	P	A	A
20.3	Results from similar studies	P	A	A	A	P	P	P	A
21	Generalizability: Discuss the applicability of the results	i	i	P	P	P	P	P	P
22	Funding: Give the source of funding	P	A	A	P	P	P	P	P
	na: not applicable	P: 31	P:29	P:29	P:30	P:32	P:36	P:33	P:30
	P: present	A:15	A:14	A:15	A:11	A:13	A:11	A:14	A:16
	A: absent	i:5	i:4	i:0	i:0	i:3	i:1	i:2	i:0
	I: incomplete	na:10	na:10	na:13	na:17	na:11	na:12	na:11	na:14
		33.5/61	31/57	29/57	30/58	33.5/59	36.5/60	34/60	30/60
		55%	54%	51%	52%	57%	61%	57%	50%
	Paper grade	Average	Average	Average	Average	Average	Average	Average	Average

		Seth A	Ihrai T	Spear S	Bonomi R	Delay E	Illouz	Rietjens M
STROBE Criteria		Retrospective	Retrospective	Retrospective	Retrospective	Retrospective	Retrospective	Retrospective
1.1	Indicate Design	P	P	P	P	P	A	A
1.2	Informative and balanced on method	P	P	P	P	P	P	P
1.3	Informative and balanced on results	P	P	P	P	P	P	P

2.1	Background: Explain background	P	P	P	P	P	P	P
2.2	Rationale	P	P	P	P	P	P	P
3	Objective: State objective	P	P	P	P	P	P	P
4	Study Design: Present key element of the design	P	P	P	P	P	P	A
5.1	Setting: Describe setting	P	P	P	A	A	P	A
5.2	Location	P	P	P	A	A	P	A
5.3	Date	A	A	P	A	P	P	A
5.4	Recruitment Period	P	P	P	A	P	P	A
5.5	Exposure	P	P	i	P	i	P	A
5.6	Follow up	P	P	A	P	P	P	A
5.7	Data collection	P	P	P	P	P	P	A
6.1	Participants: Describe criteria	P	P	P	A	A	P	P
6.2	Source	P	P	P	A	A	P	P
6.3	Method of selection	P	P	P	A	A	P	P
6.4	Method of follow up	A	A	A	P	P	P	P
6.5	Matching criteria	i	na	na	na	na	na	na
7.1	Variables: Define outcomes	P	P	P	P	P	P	A

7.2	Exposure	P	P	P	P	P	P	A
7.3	Predictors	A	A	A	A	A	A	A
7.4	Potential confounders	A	A	A	A	A	A	A
7.5	Effect Modifiers	A	A	A	A	A	A	A
8.1	For variable of interest give source of data	P	P	P	A	A	A	P

STROBE Criteria		Seth A	Ihrai T	Spear S	Bonomi R	Delay E	Illouz	Rietjens M
8.2	Details of measurements	P	i	P	P	A	A	A
8.3	Comparability method of groups	A	A	A	A	A	A	A
9	Bias: Effort to address potential bias	A	A	A	A	A	A	A
10	Study size: Describe how study size is arrived	P	P	P	P	A	A	A
11	Describe how variable were handled in analysis	A	A	A	A	A	A	A
12.1	Describe all statistical methods	P	na	na	na	A	A	A
12.2	Methods used to examine subgroups and interactions	P	na	na	na	A	P	P
12.3	Explain how to address missing data	A	na	na	na	A	na	A
12.4	Cohort study (how to address loss of follow up);	A	na	na	na	A	A	A
12.5	Describe any sensitive analysis	na	na	na	na	A	A	A

13.1	Examine and number of eligible participants	P	P	P	P	P	P	P
13.2	Completed participation	P	P	P	P	P	P	P
13.3	Follow up	P	P	P	P	P	P	P
13.4	Analysis	P	P	P	P	P	P	P
13.5	Give reasons for non-participants	P	P	P	A	na	A	P
13.6	Consider use of flow diagram	na	na	na	na	na	A	A
14.1	Characteristics of study participants	P	i	i	i	i	i	i
14.2	Information on exposure to potential confounders	i	i	A	A	i	i	A
14.3	Missing data for each variable of interest	na	na	na	na	A	A	i
14.4	Cohort study: summarise follow up time	P	na	na	na	A	na	P
15	Cohort study (report numbers of outcome event)	P	na	na	na	A	na	P
16.1	Give unadjusted estimates	P	P	P	P	P	P	P
16.2	Confounder adjusted estimates and their precision	A	A	A	A	A	A	A
16.3	Report category boundaries	na	na	na	P	A	A	P
16.4	Consider translating relative risk to absolute risk	A	A	na	na	A	A	na

STROBE Criteria		Seth A	Ihrai T	Spear S	Bonomi R	Delay E	Illouz	Rietjens M

17.1	Other analysis: Report analyses for subgroups	P	A	A	i	P	P	P
17.2	Interaction	P	A	A	A	A	A	A
17.3	Sensitivity analysis	A	A	A	A	A	A	A
18	Key results: Summarize key results with reference to study objectives	P	P	P	P	P	P	P
19.1	Limitation: Discuss limitation of the study	P	A	A	A	A	P	A
19.2	Bias	A	A	A	A	A	A	A
20.1	Cautious overall interpretation of results considering: objective	P	P	P	P	P	P	P
20.2	Limitation	A	A	A	A	A	A	A
20.3	Results from similar studies	A	P	A	P	P	P	P
21	Generalizability: Discuss the applicability of the results	P	P	P	P	P	P	P
22	Funding: Give the source of funding	P	P	A	P	P	P	P
	na: not applicable	P:39	P:31	P:29	P:33	P:24	P:28	P:35
	P: present	A:13	A:15	A:17	A:13	A:22	A:18	A:22
	A: absent	i:2	i:3	i:2	i:3	i:2	i:3	i:2
	I: incomplete	na:4	na:11	na:12	na:11	na:12	na:12	na:2
		40/58	32.5/60	30/60	34.5/60	25/58	29.5/57	36/59
		69%	54%	50%	58%	43%	52%	59%

Paper grade		Good	Average	Average	Average	Poor	Poor	Average

		Missana MC	Salgarello M	Kanchwala S	Cigna E	Delay	Longo	Gale
STROBE Criteria	TITLE AND ABSTRACT	Retrospective	Retrospective	Retrospective	Prospective	Prospective	Retrospective	Retrospective
1.1	Indicate Design	P	P	A	P	P	P	P
1.2	Informative and balanced on method	P	A	P	P	P	P	P
1.3	Informative and balanced on results	P	A	P	P	P	P	P
2.1	Background: Explain background	P	P	P	P	P	P	P
2.2	Rationale	P	P	P	P	P	P	P
3	Objective: State objective	P	P	i	P	P	P	P
4	Key element of the study design	P	P	A	A	P	P	P
5.1	Setting: Describe setting	A	P	P	P	P	P	P
5.2	Location	A	A	P	P	P	P	P
5.3	Date	P	A	P	P	P	P	P

5.4	Recruitment Period	P	A	P	A	P	P	P
5.5	Exposure	P	P	P	P	P	P	P
5.6	Follow up	P	P	P	P	P	P	P
5.7	Data collection	P	P	P	P	P	P	P
6.1	Participants: Describe criteria	P	P	P	P	P	P	P
6.2	Source	P	P	P	P	P	P	P
6.3	Method of selection	P	P	P	P	P	P	P
6.4	Method of follow up	P	A	P	P	P	P	P
6.5	Matching criteria	na	na	na	na	na	P	na
7.1	Variables: Define outcomes	P	A	P	P	P	P	P
7.2	Exposure	P	P	P	A	P	P	P
7.3	Predictors	P	P	A	A	A	A	A
7.4	Potential confounders	P	P	A	A	A	A	A
7.5	Effect Modifiers	P	P	A	A	P	A	A
8.1	For variable of interest give data source	P	A	P	A	P	P	P

STROBE Criteria		Missana MC	Salgarello M	Kanchwala S	Cigna E	Delay	Longo	Gale
8.2	Details of measurements	P	P	A	P	A	P	P
8.3	Comparability method of groups	A	na	na	na	A	P	P
9	Bias: Effort to address potential bias	A	A	A	A	A	P	P
10	Study size: Describe how study size is arrived	A	A	A	A	P	P	P
11	Describe how variable were handled in analysis	A	A	A	A	A	P	P
12.1	Describe all statistical methods	na	A	A	P	A	P	P
12.2	Methods used to examine subgroups and interactions	na	na	A	na	P	P	P
12.3	Explain how to address missing data	A	A	A	A	A	A	A
12.4	Cohort study (how to address loss of follow up);	na	na	A	na	A	A	P
12.5	Describe any sensitive analysis	na	na	A	na	na	A	A
13.1	Examine and number of eligible participants	P	P	P	P	P	P	P
13.2	Completed participation	P	P	P	P	P	P	P
13.3	Follow up	P	P	P	P	P	P	P
13.4	Analysis	P	P	P	P	P	P	P
13.5	Give reasons for non-participants	na	na	na	na	A	P	P
13.6	Consider use of flow diagram	A	P	A	A	A	na	P

14.1	Characteristics of study participants	P	P	P	P	P	i	P
14.2	Information on exposure to potential confounders	A	A	P	A	P	A	P
14.3	Missing data for each variable of interest	A	A	A	A	A	na	na
14.4	Cohort study: summarise follow up time	P	P	P	P	P	na	P
15	Cohort study (report numbers of outcome event)	P	P	P	P	P	na	P
16.1	Give unadjusted estimates	P	P	P	P	P	i	i
16.2	Confounder adjusted estimates and their precision	na	na	na	na	na	A	A
16.3	Report category boundaries	P	P	P	P	P	A	A
16.4	Consider translating relative risk to absolute risk	na	na	na	na	na	na	na

STROBE Criteria		Missana	Salgarello	Kanchwala	Cigna E	Delay	Longo	Gale
17.1	Other analysis: Report analyses for subgroups	na	na	na	na	P	P	P
17.2	Interaction	na	na	na	na	P	A	A
17.3	Sensitivity analysis	na	na	na	na	A	A	A
18	Summarize key results with reference to study objectives	P	P	P	P	P	P	P
19.1	Limitation: Discuss limitation of the study	A	P	A	A	A	P	P
19.2	Bias	A	A	A	A	A	P	P
20.1	Cautious overall interpretation of results	P	P	P	P	P	P	P

20.2	Limitation	A	A	A	A	A	P	P
20.3	Results from similar studies		P	P	P	P	P	P
21	Generalizability: Discuss the applicability of the results	P	P	P	P	P	P	P
22	Funding: Give the source of funding	A	P	A	A	P	P	P
	na: not applicable	P:31	P:39	P:30	P:33	P:36	P:43	P:48
	P: present	A:18	A:11	A:22	A:16	A:21	A:	A:10
	A: absent	i:0	i:0	i:0	i:1	i:0	i:2	i:1
	I: incomplete	na:11	na:11	na:9	na:11	na:4	na:5	na:2
		31/60	39/61	30/61	33.5/61	36/61	43/56	48/59
		52%	64%	49%	55%	59%	76%	81%
	Paper grade	Average	Average	Poor	Average	Average	Good	Good

Table 2. STROBE Assesment

STROBE Criteria	TITLE AND ABSTRACT	Petit JY Retrospective	Petit JY Retrospective	Petit JY Prospective	Beck M Prospective	Sarfati I Retrospective	Rigotti G Retrospective	Panettiere P Retrospective
1.1	Indicate Design	P	A	P	A	A	A	P
1.2	Informative and balanced on method	P	A	P	P	P	P	P
1.3	Informative and balanced on results	P	A	A	P	P	P	P

2.1	Background: Explain background	P	P	P	P	P	P	P
2.2	Rationale	P	P	P	P	P	P	P
3	Objective: State objective	P	i	P	P	P	P	P
4	Key element of the study design	P	A	P	A	A	A	P
5.1	Setting: Describe setting	P	P	A	P	A	A	P
5.2	Location	P	P	A	A	A	A	A
5.3	Date	P	P	P	P	P	P	P
5.4	Recruitment Period	P	P	P	P	P	P	P
5.5	Exposure	P	P	P	P	P	P	P
5.6	Follow up	P	P	P	P	P	i	P
5.7	Data collection	P	P	P	P	P	P	P
6.1	Participants: Describe criteria	P	P	P	P	P	P	P
6.2	Source	P	P	P	P	P	P	P
6.3	Method of selection	P	P	P	P	P	P	P
6.4	Method of follow up	P	P	P	P	P	i	P
6.5	Matching criteria	P	P	na	na	A	na	na
7.1	Variables: Define outcomes	P	P	P	P	P	P	P

7.2	Exposure	P	P	P	A	P	P	P
7.3	Predictors	P	P	P	A	P	P	A
7.4	Potential confounders	P	P	P	A	P	P	A
7.5	Effect Modifiers	P	P	A	A	P	P	A
8.1	Give data source	A	A	A	P	P	P	na

STROBE Criteria		Petit JY	Petit JY	Petit JY	Beck M	Sarfati I	Rigotti G	Panettiere P
8.2	Details of measurements	P	P	P	i	A	P	P
8.3	Comparability method of groups	P	P	P	na	na	P	na
9	Bias: Effort to address potential bias	A	A	A	A	A	A	A
10	Study size: Describe how study size is arrived	A	A	A	A	A	P	A
11	Describe how variable were handled in analysis	A	P	P	A	A	P	A
12.1	Describe all statistical methods	A	P	P	na	na	P	P
12.2	Describe any methods used to examine subgroups and interactions	A	P	P	na	na	P	P
12.3	Explain how to address missing data	P	A	A	A	A	A	A
12.4	Cohort study (how to address loss of follow up);	A	P	P	na	A	A	A
12.5	Describe any sensitive analysis	A	P	A	na	na	na	A

13.1	Examine and number of eligible participants	P	P	P	P	P	P	P
13.2	Completed participation	P	P	P	P	P	P	P
13.3	Follow up	P	P	P	P	P	P	P
13.4	Analysis	P	A	P	P	P	P	P
13.5	Give reasons for non-participants	P	P	A	na	na	na	A
13.6	Consider use of flow diagram	A	A	A	A	A	A	A
14.1	Characteristics of study participants	P	P	P	P	P	P	A
14.2	Information on exposure to potential confounders	P	P	A	P	P	P	A
14.3	Missing data for each variable of interest	A	A	A	na	A	A	A
14.4	Cohort study: summarise follow up time	A	P	P	P	P	P	A
15	Cohort study (report numbers of outcome event)	P	P	P	P	P	P	P
16.1	Give unadjusted estimates	P	P	P	P	P	P	P
16.2	If applicable confounder adjusted estimates and their precision		P	P	na	A	P	A
16.3	Report category boundaries	P	P	P	P	P	P	P
16.4	If applicable, consider translating relative risk to absolute risk	A	P	P	na	na	na	na

STROBE Criteria		Petit JY	Petit JY	Petit JY	Beck M	Sarfati I	Rigotti G	Panettiere
17.1	Other analysis: Report analyses for subgroups	A	P	P	na	na	P	P
17.2	Interaction	A	P	P	na	na	P	P
17.3	Sensitivity analysis	A	P	P	na	na	na	P
18	Summarize key results with reference to study objectives	P	P	P	P	P	P	P
19.1	Limitation: Discuss limitation of the study	P	P	P	A	P	P	A
19.2	Bias	A	A	A	A	A	A	A
20.1	Cautious overall interpretation of results	P	P	P	P	P	P	P
20.2	Limitation	P	A	A	A	P	P	A
20.3	Results from similar studies	P	A	P	P	P	P	
21	Generalizability: Discuss the applicability of the results	P	P	P	P	P	P	P
22	Funding: Give the source of funding	P	A	A	A	A	A	A
	na: not applicable	P:29	P:50	P:46	P:34	P:32	P:45	P:34
	P: present	A:25	A:9	A:15	A:12	A:18	A:12	A:21
	A: absent	i:0	i:0	i:1	i:1	i:0	i:0	i:2
	I: incomplete	na:4	na:0	na:0	na:14	na:10	na:4	na:3
		29/58	50/59	46.5/61	34.5/61	32/60	45/61	35/60

	50%	85%	75%	56%	53%	74%	58%
Paper grade	Average	Excellent	Good	Average	Average	Average	Average

Table 2. STROBE Assesment

STROBE Criteria	TITLE AND ABSTRACT	Doren	Biazus	Masia	Khouri	Brenelli	Ribuffo	Riggio
		Retrospective	Prospective	Retrospective	Retrospective	Prospective	Retrospective	Retrospective
1.1	Indicate Design	P	P	P	A	P	i	A
1.2	Informative and balanced on method	P	P	P	A	P	P	P
1.3	Informative and balanced on results	P	P	P	P	P	P	P
2.1	Background: Explain background	P	P	P	P	P	P	P
2.2	Rationale	P	P	P	P	P	P	P
3	Objective: State objective	P	P	P	P	P	P	P
4	Key element of the study design	P	P	P	A	P	i	A
5.1	Setting: Describe setting	P	P	P	P	P	P	P
5.2	Location	P	P	P	P	P	P	P
5.3	Date	P	P	P	P	P	P	P
5.4	Recruitment Period	P	P	P	P	P	P	P
5.5	Exposure	P	P	P	P	P	P	P

5.6	Follow up	P	P	P	P	P	P	P
5.7	Data collection	P	P	P	i	P	P	P
6.1	Participants: Describe criteria	P	P	P	P	P	P	P
6.2	Source	P	A	P	P	P	P	P
6.3	Method of selection	P	A	P	i	P	P	P
6.4	Method of follow up	P	A	P	i	P	P	P
6.5	Matching criteria	na	na	na	na	na	na	na
7.1	Variables: Define outcomes	P	P	P	A	P	P	P
7.2	Exposure	P	P	P	A	P	P	P
7.3	Predictors	A	A	P	A	P	P	P
7.4	Potential confounders	A	A	P	A	P	P	P
7.5	Effect Modifiers	A	A	P	A	P	P	P
8.1	For variable of interest give data source	P	P	P	A	P	P	P

STROBE Criteria		Doren	Biazus	Masia	Khouri	Brenelli	Ribuffo	Riggio
8.2	Details of measurements	P	P	P	A	P	P	P
8.3	Comparability method of groups	P	na	na	na	P	P	P

9	Bias: Effort to address potential bias	A	A	A	A	A	A	A
10	Study size: Describe how study size is arrived	P	P	A	A	A	A	A
11	Describe how variable were handled in analysis	P	P	P	A	P	P	P
12.1	Describe all statistical methods	P	na	P	P	P	P	P
12.2	Describe any methods used to examine subgroups and interactions	na	i	A	A	P	A	P
12.3	Explain how to address missing data	A	A	A	A	A	A	A
12.4	Cohort study (how to address loss of follow up);	A	A	A	A	na	na	na
12.5	Describe any sensitive analysis	A	A	A	A	P	A	A
13.1	Examine and number of eligible participants	P	P	p	P	P	P	P
13.2	Completed participation	P	P	p	P	P	P	P
13.3	Follow up	P	P	p	P	P	P	P
13.4	Analysis	P	P	p	P	P	P	P
13.5	Give reasons for non-participants	P	A	p	A	P	P	P
13.6	Consider use of flow diagram	P	na	na	P	A	A	A
14.1	Characteristics of study participants	P	P	p	P	P	P	p
14.2	Information on exposure to potential confounders	P	A	p	A	P	P	P
14.3	Missing data for each variable of interest	na	na	p	A	P	P	p

14.4	Cohort study: summarise follow up time	P	na	p	P	P	P	p
15	Cohort study (report numbers of outcome event)	na	na	p	P	P	P	P
16.1	Give unadjusted estimates	i	A	p	P	P	P	p
16.2	If applicable confounder adjusted estimates and their precision	A	A	p	A	na	na	na
16.3	Report category boundaries	A	na	p	P	P	P	P
16.4	If applicable, consider translating relative risk to absolute risk	A	na	na	na	na	na	na

STROBE Criteria		Doren	Biazus	Masia	Khouri	Brenelli	Ribuffo	Riggio
17.1	Other analysis: Report analyses for subgroups	A	A	A	P	P	P	P
17.2	Interaction	A	A	A	A	P	P	P
17.3	Sensitivity analysis	A	A	A	A	A	P	P
18	Summarize key results with reference to study objectives	P	P	P	i	P	P	P
19.1	Limitation: Discuss limitation of the study	A	i	P	A	A	A	p
19.2	Bias	P	P	A	A	A	A	A
20.1	Cautious overall interpretation of results	A	i	P	i	P	P	P
20.2	Limitation	P	P	P	A	A	P	p
20.3	Results from similar studies	P	P	P	i	P	P	P

21	Generalizability: Discuss the applicability of the results	P	P	P	p	P	P	P
22	Funding: Give the source of funding	P	P	P	P	P	P	P
	na: not applicable	P:41	P:33	P:47	P:26	P:49	P:47	P:49
	P: present	A:15	A:14	A:10	A:26	A:9	A:9	A:8
	A: absent	i:1	i:2	i:0	i:6	i:0	i:1	i:0
	I: incomplete	na:4	na:6	na:4	na:3	na:3	na: 4	na: 4
		41/57	33/55	47/57	26/58	49/58	47/57	49/57
		71%	60%	82%	44%	84%%	79%	82%
	Paper grade	Good	Average	Good	Poor	Good	Good	Good

Table 2. STROBE Assesment

STROBE Criteria	TITLE AND ABSTRACT	Rigotti	Coleman	Bezerra	Juhl	Caviggioli	Maione	Weichman	Zhu
		Retrospective	Retrospective	Prospective	Prospective	Prospective	Retrospective	Retrospective	Retrospective
1.1	Indicate Design	P	A	P	A	P	P	P	P
1.2	Informative and balanced on method	P	P	P	A	P	P	P	P
1.3	Informative and balanced on results	P	P	P	P	P	P	P	P
2.1	Background: Explain background	P	P	P	P	P	P	P	P

2.2	Rationale	P	P	P	P	P	P	P	P
3	Objective: State objective	P	P	P	P	P	P	P	P
4	Key element of the study design	P	A	P	A	P	P	P	P
5.1	Setting: Describe setting	P	P	P	P	P	P	P	P
5.2	Location	P	P	P	P	P	P	A	P
5.3	Date	P	P	P	P	P	P	P	P
5.4	Recruitment Period	P	P	P	P	P	P	P	P
5.5	Exposure	P	P	P	P	P	P	P	P
5.6	Follow up	P	P	P	P	P	P	P	P
5.7	Data collection	P	P	P	P	P	P	P	P
6.1	Participants: Describe criteria	P	P	P	P	P	P	P	P
6.2	Source	P	P	P	P	P	P	P	P
6.3	Method of selection	P	A	P	P	P	P	P	P
6.4	Method of follow up	P	P	P	P	P	P	P	P
6.5	Matching criteria	na	na	P	na	P	P	na	P
7.1	Variables: Define outcomes	P	P	P	P	P	P	P	P
7.2	Exposure	P	P	P	P	P	P	P	P

7.3	Predictors	P	A	P	P	P	P	A	P
7.4	Potential confounders	A	A	A	A	P	A	A	P
7.5	Effect Modifiers	A	A	P	A	P	P	A	P
8.1	For variable of interest give data source	P	P	A	P	P	A	A	P

STROBE Criteria		Rigotti	Coleman	Bezerra	Juhl	Caviggioli	Maione	Weichman	Zhu
8.2	Details of measurements	P	P	P	P	P	P	P	P
8.3	Comparability method of groups	P	P	A	P	A	P	P	P
9	Bias: Effort to address potential bias	A	A	A	A	A	A	A	A
10	Study size: Describe how study size is arrived	P	P	A	P	A	P	P	P
11	Describe how variable were handled in analysis	P	A	A	A	A	P	A	P
12.1	Describe all statistical methods	P	A	A	P	P	P	P	P
12.2	Describe any methods used to examine subgroups and interactions	A	A	A	A	A	A	A	A
12.3	Explain how to address missing data	A	A	A	A	A	A	A	A
12.4	Cohort study (how to address loss of follow up);	na	A	A	A	A	A	A	A
12.5	Describe any sensitive analysis	P	A	A	A	A	A	A	A

13.1	Examine and number of eligible participants	P	P	P	P	P	P	P	P
13.2	Completed participation	P	P	P	P	A	P	P	P
13.3	Follow up	P	P	P	P	A	P	P	P
13.4	Analysis	P	P	P	P	P	P	P	P
13.5	Give reasons for non-participants	P	A	A	A	A	A	A	A
13.6	Consider use of flow diagram	A	A	A	P	A	A	P	A
14.1	Characteristics of study participants	P	P	P	P	A	P	P	P
14.2	Information on exposure to potential confounders	A	A	A	A	A	P	A	P
14.3	Missing data for each variable of interest	A	A	A	A	A	A	A	A
14.4	Cohort study: summarise follow up time	P	P	P	P	P	P	P	P
15	Cohort study (report numbers of outcome event)	P	P	P	P	P	P	P	P
16.1	Give unadjusted estimates	P	P	P	P	P	P	P	P
16.2	If applicable confounder adjusted estimates and their precision	na	na	P	P	A	P	P	P
16.3	Report category boundaries	P	A	A	P	P	P	P	P
16.4	If applicable, consider translating relative risk to absolute risk	na	na	na	na	na	na	na	na

STROBE Criteria		Rigotti	Coleman	Bezerra	Juhl	Caviggioli	Maione	Weichman	Zhu
17.1	Other analysis: Report analyses for subgroups	P	A	A	A	A	A	A	A
17.2	Interaction	P	A	A	A	A	P	A	P
17.3	Sensitivity analysis	P	A	A	A	A	P	P	P
18	Summarize key results with reference to study objectives	P	P	P	P	P	P	P	P
19.1	Limitation: Discuss limitation of the study	A	P	i	P	A	P	P	P
19.2	Bias	A	P	A	A	A	A	A	A
20.1	Cautious overall interpretation of results	P	P	P	A	A	P	A	P
20.2	Limitation	A	P	A	P	A	P	P	P
20.3	Results from similar studies	P	P	P	P	P	P	P	P
21	Discuss the applicability of the results	P	P	P	P	P	P	P	P
22	Funding: Give the source of funding	P	P	P	P	P	P	P	P
	na: not applicable	P:50	P:41	P:33	P:44	P:31	P:50	P:45	P:50
	P: present	A:7	A:16	A:25	A:16	A:28	A:10	A:15	A:10
	A: absent	i:0	i:0	i:0	i:0	i:0	i:0	i:0	i:0

I: incomplete	na:4	na:4	na:3	na:1	na:2	na:1	na:1	na:1
	50/57	41/57	33/58	11/15	31/59	5/6	3/4	5/6
	84%	67%	55%	73%	52%	83%	75%	83%
Paper grade	Good	Average	Poor	Good	Poor	Good	Good	Good

2.4.3 Patient cohorts

A total of 6,046 women underwent fat grafting in the clinical literature. The average age of the patients was 49 years old. Upon review of the manuscripts, it became apparent that they all reported on four main groups of women who underwent breast cancer surgery with subsequent autologous fat grafting:

1. Breast-conserving surgery then autologous fat grafting.
2. Total mastectomy then autologous fat grafting prior to breast reconstruction.
3. Total Mastectomy and autologous flap reconstruction: deep inferior epigastric perforator (DIEP), latissimus dorsi (LD), superior gluteal artery perforator (SGAP), transverse rectus abdominis myocutaneous (TRAM), and free-TRAM reconstruction. DIEP and free-TRAM are microsurgical procedures.
4. Total mastectomy and expander/implant reconstruction with autologous fat grafting used as an adjunct procedure.

Five authors involving 1,464 patients did not specifically outline whether or not autologous fat grafting was used for women who had undergone either breast-conserving surgery or total mastectomy. Those patients were not included in the subdivision of groups below, resulting in a total of 4,582 women in all four groups (Delay et al., 2009a, Monticciolo et al., 1994, Spear SL, 2005, R, 2008, Parikh et al., 2012).

2.4.4 Breast-conserving surgery then autologous fat grafting

There were 22 papers exploring the use of fat grafting in women who had previously undergone breast-conserving surgery (BCS) (Rietjens et al., 2011, Petit et al., 1998, Petit JY, 2011, Petit et al., 2012, Beck M, 2011, Panettiere et al., 2009, Missana MC, 2007, Salgarello et al., 2011, Ihrai et al., 2013, Maione et al., 2014, Uda et al., 2014, Losken et al., 2011, IIllouz YG, 2009, Semprini et al., 2014, Kovacs et al., 2006, Uda et al., 2015, Caruso et al., 2006, Brenelli et al., 2014, Hammer-Hansen et al., 2015, Gosset J, 2008, Russe et al., 2014, Nahabedian and Galdino, 2003, Choi et al., 2013). The number of women who received fat grafting after BCS was 1,324. There was particular interest in the use of fat grafting for defect correction in the inner quadrant and décolleté area. Rietjens noted that this was often requested by women who had undergone breast-conserving surgery (Rietjens et al., 2011). Biazus injected the fat graft into the defect immediately after the breast-conserving surgery procedure in 20 patients to replace the lost tissue deficit (Caruso et al., 2006). Only one patient had fat necrosis at six months follow-up. Rietjens' study of 158 women who underwent breast-conserving surgery found one patient with recurrence at 18 months follow-up (Rietjens et al., 2011).

Petit has published three manuscripts on the subject of women who have undergone breast-conserving surgery (Petit et al., 1998, Petit JY, 2011, Petit et al., 2012). The first two earlier papers reported no increase in recurrence with a local recurrence rate of 2.1% in the breast-conserving surgery group. That rate was slightly higher than in the total mastectomy and control groups, but was not statistically significant. Although the third case-control study outlined an increased concern for women diagnosed with ductal carcinoma in-situ (DCIS) and reported a recurrence

rate of 18%, this cohort was already at high risk for recurrence. This rate of recurrence was not found in a more recent study by Gale, in which cases of DCIS and fat grafting demonstrated a recurrence rate of 0.5% (Kovacs et al., 2006).

2.4.5 Total mastectomy then autologous fat grafting prior to breast reconstruction

There were 14 papers exploring the use of fat grafting after total mastectomy, but prior to breast reconstruction (Salgarello et al., 2012, Sarfati et al., 2011, Lancerotto et al., 2013, Suga et al., 2010, Riggio et al., 2013, Rietjens et al., 2011, Petit et al., 2012, Petit et al., 1998, Rigotti G., 2010, Uda et al., 2014, Illlouz YG, 2009, Hoppe et al., 2013, Ribuffo et al., 2011, Kovacs et al., 2006, Rigotti G., 2005, Coleman, 2007, Kaoutzanis et al., 2014, Coleman S.R., 1995, Hammer-Hansen et al., 2015). The number of women who received fat grafting post total mastectomy was 1,816. By injecting the fat graft into the breast area of women who had previously undergone total mastectomy and radiotherapy, Ribuffo's study demonstrated improved skin quality and a rate of implant exposure that was reduced to zero (Ribuffo et al., 2013, Ribuffo et al., 2011). His study consisted of two groups of women who had total mastectomy and radiotherapy, one with radiotherapy and no fat grafting, and one with fat grafting after radiotherapy, but prior to expander insertion. They demonstrated a 100% success rate in a small series of 16 patients who had irradiated implant reconstruction after fat grafting, compared with a 56.25% success rate in those who were irradiated without lipofilling (Ribuffo et al., 2013). Ribuffo also noted higher rates of capsular contracture in those not treated with fat grafting prior to radiotherapy, compared with an absence of capsular contracture in those who were treated (Ribuffo et al., 2013). Longo compared 11 non-irradiated patients with 10 irradiated patients, all of whom had undergone total mastectomy (de Heras Ciechomski et al., 2012). There were no cases of local recurrence or distant metastasis, and no fat grafting-related complications after 46.15 months follow-up. However, there were differences in the quantity of fat grafting that was required to

achieve aesthetically satisfactory results. On average, 126.55 mL more fat could be injected in the group of patients who had not received radiotherapy, owing to better skin distensibility in this group. This volume difference was significant. On average, the radiotherapy group required 2.4 more procedures to achieve the same result.

Longo's group concluded that the recipient site volume determines the ratio of grafted fat volume (de Heras Ciechomski et al., 2012). In women who have had radiotherapy, only 1/3 of the mastectomy volume can be injected. Unfortunately, the exact amount of graft retention could not be quantified, as the breast volumes were not measured post-operatively (de Heras Ciechomski et al., 2012).

The longest reported follow-up period was 7.6 years, and a locoregional relapse rate of 6.5% was demonstrated in this group of patients (Rigotti G., 2010). Other large randomised control trials have reported similar recurrence rates for post-mastectomy patients (Veronesi et al., 2002). Overall, cancer recurrence rates following total mastectomy and fat grafting were comparable torates for patients who did not receive fat grafting, at between 0.4% - 0.88% per year (Kovacs et al., 2006, Riggio et al., 2013, Rigotti G., 2010).

2.4.6 Total Mastectomy and autologous flap reconstruction (DIEP, lat dorsi, superior gluteal artery perforator flaps, TRAM and free-TRAM reconstruction)

There were 16 papers exploring the use of autologous fat grafting (AFG) as an adjunct to flap reconstruction (Sinna et al., 2010, Missana MC, 2007, de Blacam et al., 2011, Seth et al., 2012, Bonomi et al., 2013, Ihrai et al., 2013, Kanchwala et al., 2009, Losken et al., 2011, Nahabedian and Galdino, 2003, Heit et al., 2012, Pierrefeu-Lagrange AC, 2006, Choi et al., 2013, Hammer-Hansen et al., 2015, Riggio et al., 2013, Suga et al., 2010, Lidell and Enerback, 2010, Cannon and Nedergaard, 2004). The number of women who received fat grafting following total mastectomy and autologous reconstruction was 784. Fat grafting was used by Delay as an adjunct to latissimus dorsi reconstruction for defect correction and to add volume. His group was able to use large amounts of autologous fat, between 200-700mL over multiple sessions. The amount of fat graft injected in flap reconstruction varied considerably. Losken used approximately 25 mL of fat in TRAM reconstruction patients for medial defect correction or in the superior pole of the breast (Losken et al., 2011), whereas both Missana and Weichman used approximately 147.24 and 147.8 mL, respectively, for defect correction (Missana MC, 2007, Lidell and Enerback, 2010). Missana specifically compared the mean volumes injected for each group. Breast-conserving surgery involved the smallest volume (75 mL), followed by implants (107 mL), then TRAM (142.14 mL), and autologous latissimus dorsi only (142.5 mL). Latissimus dorsi with an implant had the highest volume on average at 147.24 mL (Missana MC, 2007). Although the mean volumes injected for defect correction in TRAM reconstruction were lower (142.14 mL), higher volumes of 300 mL injected during repeat procedures were also

achieved (Missana MC, 2007). Zhu used an average of 176 mL during multi-site fat grafting in 10 patients without any complications (Missana MC, 2007). The injection sites included the latissimus dorsi muscle, skin paddle of the autologous flap, pectoralis major, and serratus anterior muscles. This was done concurrently with the latissimus dorsi reconstruction. He noted that this was a volume enhancing technique and although the sample size was small, the usefulness of this two-tier effect, i.e. the maintenance of an autologous reconstruction while adding volume in women without a lot of donor site adipose tissue, was apparent (Cannon and Nedergaard, 2004). Overall, the average replacement of autologous fat was half of the mastectomy specimen weight in their study.

The complication rate in Losken's group of women who underwent autologous flap reconstruction appeared to be higher than in the breast-conserving surgery group, but lower than in the implant group (Losken et al., 2011). Although these findings were not significant and possible confounding factors (e.g. radiotherapy) were not adjusted for, the author noted a general increase in complications among women who received radiotherapy. The indications for autologous fat grafting after flap reconstruction included autologous flaps that were too lateral on the chest and required the medial injection of a fat graft, and cases of free-TRAM where the flap was lower given the proximity of the internal mammary vessels and the fat was grafted at the superior pole. It can be observed that the majority of women who had latissimus dorsi, compared to those who underwent TRAM, had fat graft placed at the superior pole (79% vs. 58%, respectively). This stands in contrast to the amount of fat graft that was injected medially for LD and TRAM (10% vs. 34%, respectively) (Losken A., 2005). This suggests that the TRAM were mostly lateral, while the LDs were low, with a step-off. This

corresponds to the superior portions and medial areas injected into by Weichman in post-flap reconstruction. (Lidell and Enerback, 2010)

Kanchwala used fat grafting in TRAM patients at three months post-reconstruction as a secondary procedure for defect correction, while performing contralateral mastopexy, scar revisions, or nipple-areolar reconstruction (Kanchwala et al., 2009). Spear et al. observed the usefulness of post-TRAM reconstruction fat grafting, recognising that where dermal fillers or muscle-only latissimus dorsi flaps were often previously necessary, autologous fat grafting now provides less morbidity. Adjunctive fat grafting was required in the majority of a group of 43 women who had undergone autologous flap reconstruction (Spear SL, 2005).

Missana outlined the use of autologous fat grafting when prosthesis failure occurs within a latissimus dorsi reconstruction (Missana MC, 2007). In those cases, he removed the implant completely and replaced it with an autologous fat graft, or reduced the volume of the implant and injected a fat graft for a more natural feel (Missana MC, 2007)

2.4.7 Total mastectomy and expander/implant reconstruction with autologous fat grafting used as an adjunct procedure

There were 12 papers exploring the use of AFG as an adjunct to expander/implant reconstruction (Missana MC, 2007, Rigotti G., 2010, de Heras Ciechomski et al., 2012, Seth et al., 2012, Salgarello et al., 2011, de Blacam et al., 2011, Serra-Renom et al., 2010, Ihrai et al., 2013, Kanchwala et al., 2009, Suga et al., 2010, Losken et al., 2011, Cigna et al., 2012, Riggio et al., 2013). A total of 658 women received fat grafting following total mastectomy with implant reconstruction. Fat grafting in this patient group is useful to conceal rippling and to improve the transition between the chest wall and the implant (Losken et al., 2011). Fat grafting was used by Delay for minor defect correction secondary to either rippling in the upper part of the breast, medial area defects from rippling in the cleavage area, and lateral hollows below the mid-axillary line (Delay et al., 2009a). The amounts injected were between 50-150 mL. Missana used fat grafting for implant folds and for the softening of grade 3 and 4 contractures (Missana MC, 2007). This was also noted by Panettiere who observed a change in a patient's Baker grade from 4 to 1 after a single session of fat grafting, with no recurrence of contracture after 20.3 months follow-up (Panettiere et al., 2009). In the same paper, AFG prevented implant exposure in four cases, whereas in the control group, two cases of implant exposure could not be prevented (Panettiere et al., 2009).

In a comparative study, Seth et al. demonstrated that autologous fat grafting with silicone implants did not increase the risk of recurrence relative to women who had not undergone fat grafting (Seth et al., 2012). However, Losken noted a high incidence rate of either fat necrosis, keloid scarring, erythema, or pain in patients

who received fat grafting as an adjunct for implant reconstruction compared to the rates of complication in BCS and autologous flap reconstruction (TRAM, LD). This finding was significant.

Missana was the only author to describe the use of autologous fat grafting for failed prosthesis (Missana MC, 2007). Rather than replacing one failed implant with another implant, he offered women autologous fat grafting to fill the pocket that had been created by the implant to achieve an entire breast reconstruction using autologous fat grafting alone.

2.4.8 Pre-operative evaluation

There was great variation in the methods used for pre-operative evaluation of the women prior to autologous fat grafting. These ranged from 2D clinical photography, clinical examination, ultrasound, mammography, CT scan, and MRI scan. Two authors observed the LENT-SOMA grade of radiotherapy damaged skin in order to compare the change post-operatively (Panettiere et al., 2009, Rigotti G., 2005). Another used this measure to assess skin pliability prior to implant placement (Salgarello et al., 2012). Cigna et al. designed a non-validated Values of Aesthetic Satisfaction (VAS) scale pre-operatively then post-operatively to assess changes in patient satisfaction before and after the procedure (Cigna et al., 2012). Although this scale, similar to a Likert scale, is useful for an individual surgeon to monitor their outcomes, more effective tools to assess aesthetics are available, such as the validated BREAST-Q^{TM} questionnaire, which was disseminated in 2009. Digital photography was used with a grading scale by Longo et al. (de Heras Ciechomski et al., 2012)

2.4.9 Anaesthetic

Those manuscripts that reported on the use of anaesthesia identified that both general and local anaesthesia were required in cases of higher fat graft harvest volumes. Smaller harvests under a local anaesthetic could be done on an outpatient basis (de Heras Ciechomski et al., 2012, Rigotti G., 2010). With injection amounts of 40 to 360 mL of fat harvested from the abdomen per session, Longo et al. were able to use only local anaesthesia with sedation (de Heras Ciechomski et al., 2012). Operative time in these cases was 50 minutes (de Heras Ciechomski et al., 2012). By doing smaller volumes of fat graft on an outpatient basis, this reduced the frequency of hospitalisation and shortened recovery time.

2.4.10 Technique

A consensus exists that to improve the fat graft "take", the fat needs to be injected in aliquots (Rigotti G., 2010, Coleman, 2007, Lidell and Enerback, 2010). By injecting ribbons through subcutaneous tissue into the breast under low suction, graft retention rates are increased. Despite differences in surgeon learning curves and variables associated with patient selection, the majority of papers used the Coleman technique (Coleman, 1997).

The Coleman technique remains the most commonly used method of autologous fat harvest and injection. This method does not alter the concentration of adipose-derived stem cells (ADSCs). However, several authors no longer incorporate centrifugation, and other authors have adopted their own techniques, including Bezerra's use of a vertical decant and transfer technique which incorporates a steel sieve (Hammer-Hansen et al., 2015). Khouri et al. have designed their own equipment for fat grafting, which includes the use of a pre-operative external expansion device (BRAVA), "Khouri harvesting and injective cannulae", "Khouri Lipografter", AT-valve, tubing, and injection cannulae, and proposes superior efficacy by minimizing fat graft handling and improved sterility (Harvey et al., 2005). The benefits of these alterations to technique and equipment have yet to be explored.

The cell enrichment technique of concentrating ADSCs was less frequently cited in the articles and was not specifically investigated in this review. The principle of this technique is discussed further in chapter 7, where the co-culture of adipocytes with MCF10a and MCF7 cells in 2D and 3D microenvironments is explored.

In terms of harvest sites, the thigh may be optimal as it is less vascularised than the abdomen, with less perforating vessels resulting in less blood harvested in the aspirate. However, the abdomen can yield a higher harvest as it has greater fat deposition. Rohrich compared harvest sites and did not find a difference in yields between the abdomen and thighs (Rohrich et al., 2004). There were no studies comparing levels of patient satisfaction in terms of aesthetic yields between different areas of liposuction. Presumably, patients would be happy with the outcomes of liposuction from the abdomen and thighs, but this has yet to be investigated using a validated questionnaire or 3D measurement tool.

2.4.11 Number of Procedures

A total of 6,046 women underwent fat grafting procedures. The number of operations performed was 7,858 and the average number of procedures was 1.3 per patient. The highest number of procedures in a single patient was 10, for a total breast reconstruction (Monticciolo et al., 1994). Bezerra repeated procedures in 90% of cases (101 out of 112 patients), including one total graft loss secondary to infection (Hammer-Hansen et al., 2015). Generally, patients who had undergone radiotherapy required more procedures than those who had not undergone radiotherapy (Losken et al., 2011).

2.4.12 Average fat used per injection and overcorrection

A low volume of fat is considered less than 100 mL. A “mega volume” is considered greater than 200 mL (Monticciolo et al., 1994). Thirty-three papers reported on the average amount of fat injected per session. This ranged from 40 - 300 mL (Choi et al., 2013). The average injected volume based on all of the manuscripts was 102 mL. While Delay et al. proposed over-injecting by approximately 30% to account for fat graft resorption, few other authors described this technique. This percentage of fat overcorrection has not been reported based on fat resorption rate findings, and the BRAVA external expander device and autologous fat grafting pilot study in Chapter 5 explores fat graft retention rates further. Chapter 6 explores the measurement of fat graft retention rates using 3D laser scanners and MRI.

2.4.13 Post-operative evaluation

Again, there was wide variability in the methods of post-operative patient assessment. The majority of papers evaluated the complications associated with fat grafting based on initial clinical assessment, then imaging (ultrasound, MMG, CT scan, or MRI), with or without histological analysis by fine needle aspiration of excisions of fat necrosis and oil cysts (Missana MC, 2007). Other clinical assessments included the LENT-SOMA grading scale to observe the improvement of skin distensibility in radiotherapy-damaged tissues (Rigotti G., 2010). Most authors reported that patients received standard follow-up care for breast cancer surveillance, but that this follow-up was never specifically altered if women had undergone fat grafting. Four papers explored fat graft retention rates in post-operative evaluation (Choi et al., 2013, Monticciolo et al., 1994, Beck M, 2011, Panettiere et al., 2009). In order to assess aesthetic outcomes, the methods used were surgeon and patient opinion, panel of judges opinion, and 2D photography (Missana MC, 2007, Salgarello et al., 2012, Sinna et al., 2010, Uda et al., 2015). Post-operative assessment questionnaires included the visual analogue scale, VATS, neuropathic pain and scar assessments scores, and the DoloTest®, which is a validated measure of quality of life relating to patients' levels of pain (Lancerotto et al., 2013, Hoppe et al., 2013, Cigna et al., 2012, de Blacam et al., 2011). Scar assessment included noting the surface area of the scar, pigmentation, vascularity, pliability, and thickness (Lancerotto et al., 2013). Juhl et al. also performed pre- and post-operative punch biopsies pre- and post-grafting in six patients in order to count the number of visible dermal nerve fibres terminating in the epidermis (Lancerotto et al., 2013). They found a significantly lower number of nerve fibres at the mastectomy scar site

compared with controls. Unfortunately, they did not re-biopsy the scar site after autologous fat grafting to determine if there was any nerve regeneration.

2.4.14 Autologous Fat Grafting Alone

Whole breast reconstruction was previously limited to case reports (Howes et al., 2014, Serra-Renom et al., 2011, Babovic, 2010), though it is now being used in combination with external expander devices in order to explore improved fat graft retention rates after autologous fat grafting (Khouri R.K., 2012). Khouri et al. explored the use of autologous fat grafting alone in the context of an external expander device (Khouri R.K., 2012). The average number of repeat procedures reported in those cases was higher than when autologous fat grafting was used with other modalities, necessitating up to eight fat grafting procedures to obtain the desired volume. Unfortunately, even with the largest patient cohort, there was minimal quantitative data to observe any differences in this technique compared with the traditional Coleman technique (Coleman, 1997). Missana's use of autologous fat grafting to replace failed silicone implants also consisted of the use of autologous fat grafting only for breast reconstruction (Missana MC, 2007).

2.4.15 Fat Graft Reabsorption

Delay was the first author to suggest that there would be an autologous fat graft loss in the amount of 30% of the initial injected volume. Bonomi et al. considered this factor in his methodology when estimating the amount of fat to inject (Bonomi et al., 2013). He also added an additional 30% to account for any loss during centrifugation. Thus, his group ultimately overcorrected by 60%. Other authors took this into consideration during harvesting; Panettiere overcorrected by 10-15% and Biazus overcorrected by 30-50% (Caruso et al., 2006, Panettiere et al., 2009). However, few papers monitored fat graft resorption rates and the methods by which resorption rates are quantified. There was wide variability in the reports of fat graft loss, with one case of a total loss of a 180 mL fat graft reported by Bezerra et al., which was associated with a post-operative infection (Hammer-Hansen et al., 2015). The studies that objectively quantified fat graft loss rates included Choi et al. and Amar et al., who reported fat graft resorption rates of between 40 and 50% (Choi et al., 2013, Nahabedian and Galdino, 2003). Beck et al. analysed fat graft resorption rates over time and found the following rates of resorption: 8.9% after one month; 11% after three months; 43.5% after nine months; and 53% after three years (Beck M, 2011). Panettiere et al. and Illouz et al. suggested that the second fat graft procedure may have lower resorption rates of 20-30% (Panettiere et al., 2009, IIllouz YG, 2009). Beck asked his patients to evaluate their own levels of resorption after fat grafting at specified intervals. They reported an average resorption rate of 11.11% in the first three months, with the highest rate of 43.5% occurring between the third and the ninth months. After nine months, the rate tapered to a 53% overall graft loss. It was then measured using a 3D CT, which has been recognised as having good accuracy in breast volume measurement (Beck M, 2011). The findings of the scan

results were similar to the patient evaluations, revealing an average loss rate of 9.54% in the first three months that increased to 51.7% after nine months. Both the patient evaluations and CT scans were stable at 50% at three years. This stands in contrast to other studies that suggested reabsorption rates of 40-60% within the first four to six months.

A key limitation to the degree of overcorrection is the receptivity of the tissues to the significant amount of fat injected to replace the volume of tissue excised during the breast cancer operation. In breast-conserving surgery, injection of the volume of the excised specimen, plus an additional 30% into the defect site, may not be feasible. This suggests that repeated sessions of fat grafting might be more beneficial than initial over-grafting. At smaller volumes, the take of the graft may be higher with an overall net benefit of reducing the risk of haemorrhage from abdominal liposuction, fat necrosis, and decreased donor and recipient site morbidity.

Whether there are differences in fat graft absorption between groups of women who have undergone fat grafting post breast conserving surgery, total mastectomy (with and without reconstruction) is an area that warrants further investigation. Fat graft retention rates may be higher when injected into women who have remaining breast tissue (women post breast conserving surgery) or in women who have undergone breast reconstruction and have vascularised flaps in situ.

2.4.16 Minor Complications

In most cases, the papers that discussed several different treatment groups, i.e. breast-conserving surgery vs. total mastectomy, did not discuss complications separately. Thirteen studies reported minor complications including infections at the harvest site, which were treated with oral antibiotics on an outpatient basis (Rietjens et al., 2011, Petit JY, 2011, Sinna et al., 2010, Missana MC, 2007, Seth et al., 2012, Spear SL, 2005, Bonomi et al., 2013, Ihrai et al., 2013, Uda et al., 2014, IIllouz YG, 2009, Russe et al., 2014, Caruso et al., 2006, Robinette, 2002). These minor complications did not seem to affect the outcome of autologous fat grafting. Losken also included keloid scarring in his complications list, but this is not considered a specific complication of autologous fat grafting (Losken et al., 2011).

2.4.17 Major complications

Fat necrosis was reported in 24 studies reported involving 3,347 women who had undergone autologous fat grafting (Rietjens et al., 2011, Petit JY, 2011, Sinna et al., 2010, Missana MC, 2007, Seth et al., 2012, de Blacam et al., 2011, Delay et al., 2009a, Spear SL, 2005, Bonomi et al., 2013, Uda et al., 2014, Losken et al., 2011, R, 2008, Parikh et al., 2012, Cigna et al., 2012, Gosset J, 2008, Hoppe et al., 2013, Pierrefeu-Lagrange AC, 2006, Uda et al., 2015, Caruso et al., 2006, Monticciolo et al., 1994, Coleman S.R., 1995, Hammer-Hansen et al., 2015, Brenelli et al., 2014, Kaoutzanis et al., 2014). There were 253 cases of fat necrosis in 5,353 procedures resulting in an overall fat necrosis rate of 4.7%. Doren et al. reported requiring a tissue diagnosis in 17 patients out of 64 who had palpable post-operative masses (Uda et al., 2015). Fourteen patients were needle biopsied, with one core biopsy and two excisional biopsies. Parikh biopsied 22 out of 66 areas of fat necrosis, but that paper did not explore the reasons for an increased fat necrosis rate. In all cases, the biopsies were negative for recurrence (Parikh et al., 2012).

Unfortunately, the size dimensions of the fat necrosis were unspecified, and they are necessary to specifically apply the Rao and Saag criteria (Howes et al., 2014). It may be presumed that in the two cases of excisional biopsies, the fat necrosis was likely greater than 3 cm in size and tender to palpation. Seroma was reported in five out of 3,673 cases, resulting in an incidence rate of 0.13%.

Pneumothorax was reported in seven cases, the majority of which were from a single paper (Monticciolo et al., 1994, Sinna et al., 2010). There were no reports of stroke from intravascular injection of fat with subsequent emboli, and no reports of blindness (Kovacs et al., 2007, Tepper et al., 2008).

2.4.18 Interference with radiology

There were no papers that identified a difficulty in distinguishing fat necrosis from malignancy. In order to ensure patient safety, the majority of investigators identified a need to further examine necrotic fat lesions with a histopathological diagnosis (Parikh et al., 2012, Uda et al., 2015). Injecting too great an amount of fat graft can lead to increased risk of fat necrosis, since the centre of the graft lacks vascularity and nutrient supply, and thus cyst formation occurs, along with calcifications. Increased incidences of fat necrosis in patients who received larger volumes of injected fat graft were identified in Costantini's paper, though the finding was not significant (R, 2008). On imaging, he noted complex masses with internal colliquation that had a radiolucent centre and course calcifications around the margin. In four out of 77 patients in Rietjen's paper, interference with radiology was observed, but the lesions were eventually determined to be benign (Rietjens et al., 2011). It is conclusive from the literature that fat necrosis and calcifications are easily identifiable using multi-model imaging including ultrasounds, mammogram, or MRI (Lidell and Enerback, 2010, R, 2008, Howes et al., 2014).

2.4.19 Radiotherapy and skin regeneration

Several papers reported on the association between radiotherapy and fat grafting (de Heras Ciechomski et al., 2012, Panettiere et al., 2011, Rigotti G., 2005, Salgarello et al., 2012, Serra-Renom et al., 2010, Maione et al., 2014, Lancerotto et al., 2013, Losken et al., 2011). In Rietjen's paper, six out of seven patients with the complications of fat necrosis, cellulitis, or in one case a large abscess, had also undergone radiotherapy (Rietjens et al., 2011). Surgical techniques and tissue quality may influence the complication rate (Rietjens et al., 2011). Losken noted that the women who required a larger fat graft were those with prior radiotherapy, due to the subsequent loss of compliance of the overlying tissue (Losken et al., 2011). In that group, twice as many procedures were necessary to achieve the same results as the non-radiotherapy patients, and this finding was significant. However, complication rates were not higher in women who had undergone radiotherapy..

Along a similar principle of regeneration, Delay noted that fat grafting seemed to reduce the amount of capsular contracture, although this was not specifically investigated in his study (Delay et al., 2008). Juhl et al. and Caviggioli et al. both noted improved surgical scar appearance after fat grafting in women who had undergone total mastectomy (Lancerotto et al., 2013). Maione et al. found a significant improvement in radiotherapy-related pain after fat grafting in women who had undergone breast-conserving surgery (Maione et al., 2014). Hoppe used several multiple small graft injections to resolve radiation damage, and in some cases it was used as a salvage procedure when expander/implant reconstruction failed (Hoppe et al., 2013).

In autologous flap reconstruction, Zhu demonstrated no increased complications after injecting fat graft into the latissimus dorsi muscle and skin paddle in women who had previously undergone radiotherapy (Cannon and Nedergaard, 2004).

2.4.20 Recurrence rates

Twelve papers investigated cancer recurrence rates during follow-up periods ranging from one month to 12 years (Riggio et al., 2013). Generally, since the papers that reported on recurrence used different definitions of local recurrence, it was challenging to make satisfactory comparisons. Some referred to local recurrence as the diagnosis of a new lesion during the follow-up period in the same quadrant of the breast as the initial lesion. However, there was little specificity as to whether "local" included all regional recurrences involving axilla, internal mammary, and/or infra-clavicular nodes. Cases of higher recurrence were noted in patients already considered to have a high risk of recurrence, irrespective of the use of autologous fat grafting (Petit JY, 2011). Riggio et al. had the longest follow-up period (12 years) in his mixed cohort study (BCS, total mastectomy, total mastectomy with flap reconstruction), with a recurrence rate of 5%, which was comparable to Rigotti's 5.9% recurrence rate. However, both of these results are comparable to the non-fat graft groups (Riggio et al., 2013, Rigotti G., 2010).

2.4.21 Time between mastectomy and fat grafting

The longer the timespan between mastectomy and fat grafting, the greater the likelihood of that no recurrence relating to the introduction of ADSCs will occur (Kovacs et al., 2006). The majority of manuscripts did not include the gap between mastectomy and fat grafting. Ribuffo et al. described the use of autologous fat grafting post-mastectomy and prior to radiotherapy treatment in order to protect women from implant extrusion (Ribuffo et al., 2013). In his study, there were no local recurrences at 18 months follow-up. The two recurrences encountered in Ihrai's treatment group were noted 7.7 and 13.2 months post breast cancer surgery (Ihrai et al., 2013). Although both patients were at high risk of recurrence, as one had a large tumour and the other had pectoralis major muscle involvement, Ihrai concluded that a waiting period of three years was suitable in high risk patients.

2.4.22 Discussion

Clinical literature on autologous fat grafting

Upon reviewing the literature on autologous fat grafting in women who have previously undergone mastectomy for breast cancer it's the benefits of using this procedure as an adjunct to breast reconstruction are evident. Since Spear et al. re-introduced fat grafting for use in patients undergoing breast reconstruction for breast-conserving surgery and total mastectomy, a large body of evidence has emerged in support of the use of autologous fat grafting in this set of patients (Spear SL, 2005). Clinically, its use is varied, ranging from minor defect correction to whole breast reconstruction using only autologous fat grafting (Howes et al., 2014, Missana MC, 2007). The Coleman technique is the method preferred by most surgeons, with incorporation of occasional modifications aimed at improving the take of the fat graft. Initially, centrifugation was a component of the technique, but as it has been shown to be more destructive to adipocytes, it appears to be used less often than in earlier studies (Rohrich et al., 2004). In the authors experience using centrifuge in Chapter 7, the higher revolutions per minute on the centrifuge resulted in a greater volume in the oil layers. Presumably this is from the destruction in adipocytes.

The type of anaesthesia varied depending on the aim of the fat graft harvest and the type of injection. Smaller amounts were harvested using only local anaesthetic, while larger amounts were harvested under general anaesthetic. There are no guidelines specifying which fat graft amount corresponds to the use of general rather than local anaesthetic. The author assumes this would be for harvest volumes greater than 100 mL, but this is yet to be investigated.

The amount of fat grafted and the number of procedures corresponded to both the indication and the level of fat graft loss as a complication of fat grafting.

Smaller volumes were used when the intention of the surgeon was for patients to undergo further breast reconstruction procedures, or for either small defect correction post breast-conserving surgery or autologous flap reconstruction. Higher volumes were used when fat grafting was to serve as the primary method of reconstruction, and the surgeons were more concerned with fat graft loss rates in those cases. In general, the literature was inconclusive about the progression of fat graft loss within the first nine months, and it seemed to be related to the individual patient, surgeon, and use of 3D volume scanning. In Choi's retention volume study, a dose-dependent relationship between the volume of injected fat and how the degree of retention was identified (Choi et al., 2013). Overall, higher volumes of injected fat lead to higher volumes of fat retention. They also compared the percentage of fat retained between the groups in their study (lumpectomy, implant, and autologous), and found greater retention rates in the lumpectomy group compared to those in the implant or autologous groups. This finding was not significant, but it would be expected that the autologous group might have higher retention rates than the expander/implant group as fat graft is more likely to survive when adjacent tissue is native breast tissue. Smaller volumes injected into the lumpectomy area would be hypothesised to have superior retention, and this did indeed prove to be the case. Generally, the type of breast cancer surgery determined the amount of fat that could be injected in the first operation. In women who underwent total mastectomy with subsequent autologous fat grafting, Longo noted that he was able to replace 1/3 of the total mastectomy weight (de Heras Ciechomski et al., 2012). In contrast, Zhu was able to replace 1/2 of the mastectomy weight when injected into an autologous flap (Cannon and Nedergaard, 2004). Fat graft retention rates and the efficacy of autologous fat grafting in combination with external expander devices are further explored in Chapter 5. The theory proposed by Costantini that increasing volumes result in

increased likelihood of fat necrosis, along with the need to increase the necessary fat graft amount in radiotherapy patients, remain complex problems faced by plastic surgeons (R, 2008). Since the recipient site lacks vascularity and blood supply, this lack of support for transplanted fat could result in an increase in fat necrosis, as well as a lack of skin turgor and rigidity (Losken et al., 2011), leading to a significant increase in intra-compartment pressure. One corollary of the injection of fat into this environment is that it might improve local vascularity and skin turgor. The damaging effects of radiation on tissue have been classified as immediate, intermediate, and late (Dolderer et al., 2007). One potential immediate effect on the breast is oedema, involving the engorgement of intra-mammary lymphatics and ducts. In the intermediate phase, the immediate effects may cause intimal arterial damage, breast fat necrosis, and non-purulent inflammation. In the later stages, the breast can eventually fibrose, undergo glandular atrophy, decreased oxygen and arterial supply of tissues, and non-pliability. Secondarily, it can induce malignancies, lymphoma, angiosarcoma, and invasive ductal carcinomas. Specific to breast reconstruction, women who have previously undergone radiotherapy may have an increased risk of infection, implant exposure, autologous flap reconstruction failure, capsular contracture, and prosthesis exposure from flap thinning. Given the skin changes and the hypoxic environment, Choi identified a lower fat graft retention rate, but this finding was not significant (Choi et al., 2013). The corollary to the potential risk of injecting fat into women who have previously undergone breast cancer surgery is that adipose derived stem cells (ADSCs) can potentially survive in hypoxic environments and actually re-vascularise these tissues. This has been assessed clinically by observing changes in the LENT-SOMA grades by Panettiere, which showed that with repeated episodes of fat grafting, there were observable changes in the long-term effects of radiotherapy (Panettiere et al., 2009). The optimal approach

for patients who have undergone radiotherapy could therefore include smaller amounts of fat injected over a greater number of sessions.

Complications varied from minor infections to serious complications such as pneumothorax. The most concerning complication of AFG was pneumothorax, which was not investigated in-depth by any author. Presumably, the mechanism is a puncture of the intercostal muscles that opens a one-way valve from the injection cannulae. This serious complication should be described to women during the informed consent process. Moreover, the mechanism of this complication needs further examination in order to prevent this complication. When exploring the effects of different complications on different groups of patients (BCS, total mastectomy, flap reconstruction, implants), it is difficult to correlate any specific difference in the complication rates between these groups owing to poor reporting in the manuscripts.

Indications for autologous fat grafting

Some interesting indications for fat grafting included the treatment of persistent pain post breast cancer treatment (PPBCT) (Caviggioli et al., 2011, Lancerotto et al., 2013, Maione et al., 2014). Klinger et al. were the first to note that fat can have "healing" properties when he used fat injected into the face of a patient with severe burns, with skin biopsies taken pre- and post-grafting (Klinger et al., 2008). Increased angiogenesis from recruitment of endothelial progenitor cells was noted. This finding was also noted by Rigotti among women who had undergone breast cancer irradiation (Rigotti G., 2005). Another theory proposed by Gaetani et al. suggested that adipose-derived-stem-cell-mediated loose connective tissue regeneration and architectural remodelling occurs after fat graft placement (Dolderer et al., 2007). In the three articles that discussed the use of fat grafting for alleviating pain, it was clearly indicated for use in instances of pain after both breast-conserving

surgery and total mastectomy (Lancerotto et al., 2013, Klinger et al., 2008, Rigotti G., 2005). The discomfort in the breast-conserving surgery cohort described by Maione was more likely due to post-radiotherapy skin damage, rather than due to direct damage to nociceptors incurred by the sharp dissection of the skin during mastectomy, which requires longer incisions than does breast-conserving surgery (Maione et al., 2014). According to Juhl et al., patients who had experienced persistent post-mastectomy pain, which was roughly 24-25% of the women, were randomized into either a fat grafting or no intervention group. Using a visual analogue scale, neuropathic pain symptom inventory, and patient and observer scar assessment scales, fat grafting was shown to lead to significant improvements in health-related quality of life and scar appearance. This corresponds to the findings of the cohort study described in Chapter 4 of this thesis, in which an examination of post-mastectomy quality of life revealed increased incidences of pain in the chest area in women who had undergone breast-conserving surgery (Cronin et al., 2004). Other indications included improving radiotherapy damage prior to expander/implant reconstruction, defect correction in women who had undergone breast-conserving surgery, adjunctive treatment for additional volume in women who had undergone more traditional methods of breast reconstruction (flap reconstruction), and protection against implant extrusion (Ribuffo et al., 2013).

Autologous flap reconstruction and autologous fat grafting

Although autologous flap reconstruction has been deemed reliable and reproducible, with DIEP reconstruction considered the gold standard, there were several reports of the usefulness of autologous fat grafting as an adjunct to modify defects and to add volume. In some instances, this could prevent the need for the addition of silicone implants and maintain the autologous nature of the

reconstruction. In this review, the number of patients in the literature in which fat grafting was used as an adjunct to flap reconstruction was comparable to the number in which it was used as an adjunct to silicone implant reconstruction. The indications were the same: to improve volume, correct defects in order to improve rippling, and improve symmetry. Both flap reconstruction and implant reconstruction had "step-off" deformities at the superior portion of the reconstruction that required an injection of fat graft to improve the gradient from chest wall to breast. Although patients with poorer outcomes associated with autologous or implant-based reconstructions sought the addition of fat grafting, there are also women who do not wish to have either autologous or implant-based reconstruction who instead opt for only autologous fat grafting procedures, which is discussed further in chapter 5. The use of autologous fat grafting for rippling and defect correction was outlined by Losken et al., who also used fat grafting in an autologous flap reconstruction patient (Losken et al., 2011). He observed a significant increase in complications associated with his implant fat grafting group. Although the author did not specifically discuss this finding, it could be assumed that fat injected into a well-vascularised flap may have a higher take rate than when it is injected adjacent to a silicone implant. This finding would need to be confirmed in a larger study cohort. Missana used autologous fat grafting in isolation to aid failed prosthetics or when prostheses had been used with latissimus dorsi reconstruction. He replaced the prosthesis with autologous fat grafting, resulting in whole breast reconstruction with fat grafting within the pocket that was created by the implant (Missana MC, 2007).

Autologous fat grafting procedures and patient satisfaction

Generally, the satisfaction of patients seemed to be high once their treatment was complete, although this has been poorly documented and has rarely been

objectively or quantifiably measured using a validated measuring tool. The maintenance of the grafted fat volume over time also has yet to be investigated using a validated outcome measurement tool. However, it appears that fat grafting has an established niche in the domain of plastic and reconstructive surgery, in that it can help provide patients with optimal, desired results and ameliorate some of the consequences of breast cancer treatment. There are important considerations when introducing adipose derived stem cells into an area in which excision of the primary tumour has not been completed. The appropriate timing for adjuvant treatments such as fat grafting following the completion of breast cancer treatment has yet to be determined.

Autologous fat grafting and oncogenesis

There is on-going concern regarding the oncogenic potential of fat grafts and tumour recurrence rates. Proposed theories include oncogenesis derived from the paracrine or apocrine influence of adipose derived stem cells on quiescent breast cancer cells, and the limited positive findings in animal models have perpetuated the fear of fostering a similar mechanism of oncogenesis in women. Although this fear is not unfounded, it is discordant due to a lack of current support from clinical evidence, which has been outlined in the current review. Upon review of the patient cohorts in these manuscripts, there appeared to have been a higher number of patients who underwent total mastectomy and fat grafting compared with the number of patients in the breast-conserving surgery group, excluding those patients who had fat grafting as an adjunct to either autologous flap or implant reconstruction. This may account for the overall lower rates of breast cancer recurrence in women who have received fat grafting. However, on subgroup analysis, there did not appear to be higher rates of recurrence in the breast-conserving surgery group. In Biazus' cohort

of patients who underwent immediate fat grafting post breast-conserving surgery conducted by oncoplastic surgeons. The issue may be that they will not have histopathological margins of the resected tissue specimen, nor would the women have completed their adjuvant therapy for breast cancer. Therefore, .it would be interesting to calculate the recurrence rates in this patient group over the next five years, as the addition of stem cells while breast cancer cells may remain in situ, with unknown clearance of margins, is inherently risky (Caruso et al., 2006).

An increase recurrence of breast cancer in women with previously resected DCIS who then underwent autologous fat grafting was demonstrated by Petit and his team and that paper has provoked the most discussion(Petit et al., 2012). His retrospective case-controlled cohort study used the European Institute of Oncology (IEO) Breast Cancer database to identify 59 women with post-reconstruction fat grafting compared against a control group of 118 women who underwent quadrantectomy without fat grafting. These groups were followed up post breast cancer surgery for a period of 63 and 66 months, respectively. Patients were matched by age (within five years), type of surgery ("quadrantectomy"), DCIS, receptor status, and radiotherapy status. Local recurrence rates were six in the fat grafting group and three in the control group, yielding five-year cumulative incidence rates of 18% and 3%, respectively ($p=0.02$). Interestingly, the expected cumulative incidence in the control group should have been 1% per year. During subgroup analysis, the author honestly observed that the higher recurrence rates may have related to increased risk factors in the following subgroups: patients less than 50 years of age, high grade of disease (Bloom and Richardson grade 3), high Ki-67 > 14, and questionable resection margins (three patients with resection margins <1mm). They also noted that margin status was not matched between the treatment

and control groups. Margin status is defined as the pathological width of normal breast tissue around the cancer used to determine whether all of the cancer has been surgically resected. The majority of patients in both groups were oestrogen/progesterone receptor positive so we assume that those patients were treated with hormonal therapy. The authors do not clarify if patients received hormonal therapy or if they were still under hormonal therapy at the time of relapses. This paper contrasted their earlier work that compared 321 patients who underwent fat grafting with a control group of 642 patients who did not undergo fat grafting. There was no statistical difference in the rates of local recurrence between the two groups (hazard ratio, 1.1; 95% confidence interval, 0.47-2.64; p=0.79). Likewise, Gale compared 211 patients who underwent mastectomy and subsequent fat grafting with 422 patients who did not undergo fat grafting, and again there was no finding of increased recurrence (Kovacs et al., 2006). Nevertheless, patients with DCIS should be considered for breast MRI, as ultrasound and mammography do not always capture this disease, and it should include contrast to identify the increased vascularity of DCIS.

Follow up after autologous fat grafting

Follow-up periods for cancer recurrence should be a minimum of two years and should include the patient's tumour characteristics (Haloua M H, 2013). Petit's paper was well-designed when cross-checked with STROBE data (Petit JY, 2011). However, It was difficult to compare case-control study patients, as the treatment group of women who had undergone mastectomy for breast cancer were mixed lumpectomy and total mastectomy patients. And within these two subgroups of women, they had different risk status higher risk subgroups being unadjusted. This may have increased the effect estimation precision. It was then difficult to establish a

causal hypothesis for cancer recurrence. Masia et al. observed equivalent recurrence rates of 3% in women who have had autologous flap reconstruction (Heit et al., 2012). Interestingly, Ihrai injected fat graft into patients who underwent breast conservation, latissimus dorsi reconstruction, and implant reconstruction (Ihrai et al., 2013). The only patients who experienced recurrence were two patients who underwent total mastectomy and implant reconstruction using autologous fat grafting as an adjunct. That paper refuted the earlier claim that fat graft and flap reconstruction used together may result in an increased cancer recurrence rate. Unfortunately, there was a lack of patient demographic information pertaining to whether or not they were at increased risk of breast cancer recurrence.

The work up for a patient's risk should include a detailed history including age and history of cancer both in the individual and in the family, and the woman's histology should be reviewed with reference to the tumour grade, size, receptor status, lymphovascular or nodal involvement, and the tumour clearance. Sanghani et al. validated a web-based predictive nomogram for women at risk of breast cancer within 10 years, and this could be used as part of pre-operative assessments of a woman's suitability for autologous fat grafting (Krumboeck et al., 2013).

Quality of the literature on autologous fat grafting

Upon review of the article designs using the NHMRC guidelines and STROBE checklist, there was only one randomised control trial. Unfortunately, the article included a very small number of participants, rendering it prone to bias. The majority of the remaining papers on autologous fat grafting were retrospective in nature and those that were prospective have limited follow-up periods given that this method has only been used in this patient population in the last two decades. The STROBE statement is a tool used to assess the quality of epidemiological studies that

has been employed by journals to improve manuscript design, e.g. JPRAS. Results obtained from well-designed observational studies can yield beneficial information, which can then support higher levels of evidence-based research in the future. Although the majority of manuscripts were poorly designed, the efficacy and increasing use of autologous fat grafting is evident. However, there remains an overall paucity of well-designed studies with appropriate methodology, outcomes assessments, and follow-up periods. Systematic bias relating to errors in design, recruitment, data collection, or analysis could result in mistaken estimations of the true effect of fat grafting on complications and recurrence outcomes. In terms of the European perspective, the French remain reticent about the use of fat grafting in previous breast cancer patients. In 2007, the French Society of Plastic, Reconstruction, and Aesthetic Surgeons cautioned that fat grafting should only be performed in the context of a prospective controlled protocol. Currently the American Society of Plastic Surgeons have been attempting to answer questions of the FDA with regard to minimal manipulation of fat grafting for the use in breast reconstruction. Currently in Australia there is no Medicare Benefits Schedule (MBS) subsidising the use of autologous fat grafting in the public system. It can only be utilised in the context of research studies governed by human research and ethics committee oversight.

Unfortunately, very few authors responded to the request for further information for this review, and this hampered the quality of reporting in this study. Owing to the overall poor quality of reporting in these studies, there is the potential for different risk estimates in the presence of effect modification, confounding, and bias. Although previous systematic reviews have sought to answer the question of recurrence by using the collective reporting of case-control studies, these papers did not include an experimental approach, did not restrict and filter for particular groups,

lacked patient matching, and therefore the results analysis was neither stratified nor adjusted. Experimental studies that are designed as observational studies involving level 2 or level 3 evidence in the surgical literature can be critically important when it comes to establishing new techniques (Song and Chung, 2010). In this review, the papers that demonstrated both increased risk and no risk were retrospective in nature.

Another limitation of this review study is that when authors did not specify characteristics about their manuscript, e.g. whether it was retrospective in nature, this had to be determined by the investigators. It was therefore open to interpretation and discussion, which potentially introduced an element of investigator bias. However, by using two main investigators and a third in case of disagreement, this potential was minimised.

Nonetheless, a few things are certain when reviewing the literature. The current clinical research is rife with diametrically opposed opinions regarding the safety of fat grafting in this set of patients. Given the lack of evidence and the recent interest in this procedure, the only consensus is that there need to be further studies investigating its safety. The corollary principle established by this review is that those patients who have been selected to undergo autologous fat grafting, who are considered at high risk of breast cancer recurrence, and who have not had a period of disease-free survival after completion of breast cancer treatment are likely to have higher rates of local disease recurrence.

Fat grafting for breast reconstruction has been used in this patient cohort for 17 years. Its benefit is evident, not only for breast reconstruction, but for scar management and healing of radiotherapy damage to soft tissues. In contrast to the irrefutable benefits of its use in women who have had breast cancer surgery, there is no convincing evidence of increased rates of oncogenesis. Overall, there appear to be

lower recurrence rates in patients who undergo autologous fat grafting post breast cancer surgery. Since fat grafting is employed with great caution in breast cancer patients, its use in this cohort requires a multi-disciplinary approach, including discussion during combined meetings with medical oncologists, breast and endocrine surgeons, plastics surgeons, pathologists, and radiologists. In this way, it may be possible to prospectively monitor these patients in a tertiary centre with an appropriate follow-up period of greater than five years. Regarding efficacy, the current study has demonstrated the encouraging success of the procedure in women who have undergone breast conservation and total mastectomy. In principle, this technique can be suitable for breast reconstruction and should be considered along with other options: autologous flap and expander/implant reconstruction. All of these techniques aim to improve breast volume, shape, contour, deformity correction, and projection. Unique to autologous fat grafting is the ability of ADSCs to improve skin pliability post breast cancer surgery, though the level 111b evidence suggests a need for high quality prospective randomized control trials conducted scientifically and presented in formats comparable to those set out in the STROBE guidelines. This will enable an expansion of our understanding of the safety and efficacy of this technique.

Chapter 3. Quality of Life following total mastectomy with and without reconstruction vs. breast-conserving surgery for Breast Cancer: A case-controlled cohort study*

* A condensed version of this study was published: HOWES, B. H., WATSON, D. I., XU, C., FOSH, B., CANEPA, M. & DEAN, N. R. 2016. Quality of life following total mastectomy with and without reconstruction versus breast-conserving surgery for breast cancer: A case-controlled cohort study. J Plast Reconstr Aesthet Surg, 69**,** 1184-91.

3.1 Introduction

Breast-conserving surgery followed by radiotherapy is the current standard of care for most small breast cancers (NHS, 2011). Evidence for this approach has been established by various trials, including the United States National Surgical Adjuvant Breast trials (NSABPB04, NSABPB06), which have demonstrated equivalent survival outcomes (Fisher et al., 1995, Fisher et al., 1999) although quality of life was not evaluated in these trials. Recent reports have shown that an increasing proportion of women with breast cancer are actually choosing mastectomy and reconstruction.(Albornoz C, 2012) Whether this option yields good quality of life outcomes has not been established.

The development of oncoplastic surgical techniques (Fosh et al., 2014, Clough and Baruch, 1992) and the increased awareness of genetic risks for breast cancer (Metcalfe et al., 2008) are factors that might be impacting current treatment patterns in women at high risk. Albornoz et. al.(Albornoz C, 2012) recently hypothesised that

testing for BRCA mutations is contributing to an increasing rate of bilateral mastectomy and reconstruction in women with breast cancer. What is not known, however, is whether this type of surgery can deliver as good a quality of life outcome as breast-conserving surgery and radiotherapy. In addition, only a few studies have compared patient-reported outcomes following breast-conserving surgery vs. mastectomy with and without reconstruction. (Al-Ghazal et al., 2000, Nicholson et al., 2007)

To evaluate this further, the current study was conducted to evaluate quality of life outcomes in a group of women with no history of breast cancer, women who had undergone breast-conserving surgery, women who had undergone total mastectomy without breast reconstruction and women who had undergone total mastectomy with breast reconstruction. The results of this study will help establish whether there is a role for autologous fat grafting for breast reconstruction amongst these groups of women to improve quality of life.

3.2 Methods

3.2.1 Ethics Approval Number

This study was approved by the Southern Adelaide Clinical Human Research Ethics Committee (approval numbers 320.13 and 354.13) and conducted in accordance with the STROBE guidelines. All patients were given patient information sheet (Appendix 5) and as well as standard operative consent, were also given a consent for the purpose of research (Appendix 6).

3.3 Recruitment

Pre-existing prospectively collected quality-of-life datasets were available for the two total mastectomy groups, and these datasets were analysed for this study. These data were collected prospectively from 1 January 2009 to 31 December 2013 when these women attended the Flinders Breast Reconstruction Service at Flinders Medical Centre, Adelaide, South Australia. At the clinic visit, women completed the BREAST- Q^{TM} questionnaire, a previously validated, disease-specific quality-of-life measure. Scores were collated and stored in a database. For these groups, demographic and BREAST-Q^{TM} data were extracted from the database.

For this study, additional data were collected from the other two groups. Women who had undergone a single-sided breast conservation operation for cancer at Flinders Medical Centre from 1 January 2009 to 31 December 2013 were identified from multi-disciplinary team meeting records and invited to participate in the current study. Patients who had died, those who had proceeded subsequently to total mastectomy, those who had undergone reconstructive surgery after breast conservation, and those who had undergone a bilateral procedure were excluded. These patients were mailed an explanatory letter, the BREAST-Q^{TM} patient-reported outcomes measure, a study-specific adjunct questionnaire, and a reply-paid envelope for return of the questionnaire.

A control group of women (aged 20 to 87 years) was recruited from nurses and volunteers at Flinders Medical Centre. Nursing co-ordinators and the president of the Hospital Volunteers Service assisted in recruitment by placing notices explaining the study in communal areas, along with the questionnaires and instructions for their return.

The study-specific questions asked whether patients thought they had asymmetry between their two breasts following surgery and whether they would consider surgical remediation for any asymmetry. To ensure individuals with breast cancer were not included in the control group, the control version of this questionnaire also asked whether they had had breast cancer surgery. If a questionnaire was not returned, it was followed up with one telephone call. For the post-reconstruction group, data were extracted from the same source. Demographic information, details of surgery and histopathology were obtained from hospital records for all groups.

3.3.1 Groups of Recruited Patients

A case-controlled cross-sectional study was conducted, comparing four groups of women:

1. controls - women who had never been diagnosed with breast cancer or had breast cancer surgery.

Women who had undergone:

2. breast-conserving surgery for breast cancer;
3. total mastectomy without breast reconstruction;
4. total mastectomy and breast reconstruction procedure(s).

3.3.2 The BREAST-Q^{TM}

The BREAST-Q^{TM} is a validated patient-reported outcome measure developed by the Memorial Sloan Kettering Cancer Institute and the University of British Columbia. It contains 36 questions, and the raw scores are converted to summary scores out of 100 for three satisfaction and three well-being domains using "Q score" software (Pusic, A. Q Score Software 2003, https://webcore.mskcc.org/breastq/scoring.html). In this study, the domains of "Satisfaction with Breast", "Physical Well-being Chest", "Psychosocial Well-being" and "Sexual Well-being" were evaluated (Pusic, 2009). Patients who answered less than half the items within each domain were excluded from analysis. The "Physical Well-being Chest" domain encompasses pain symptoms and tightness in the upper limb, shoulder girdle and chest area and a higher score denotes less pain and discomfort than a lower score. The questionnaire

enables the translation of the patient's subjective experience in various domains to a numerical score that can then be used for quantitative analysis.

3.3.3 Statistical Methods

Prior to commencement of the study, a power calculation was performed based on previously collected Flinders Breast Reconstruction Database BREAST-Q^{TM} data. The "Satisfaction with Breast" domain was used as the index parameter (range = 0 to 100). Based on this previous experience, a difference of 10 points (10%) was thought to be clinically important and a difference of 20 points (20%) was thought to be very clinically important. This assumption matches the developers' (https://webcore.mskcc.org/breastq/qscore/qscore-manual). To detect a difference of 20% for satisfaction between the four groups, it was determined that a total of 237 participants (79 per group) would be needed. This would also include evaluating a magnitude of difference of 10% between any pair of scores within a group (i.e. scores within the breast conservation group) with the "Satisfaction with Breast" domain, set at a 2-sided Type 1 error rate of alpha=0.05 (PASS software, NCSS, Utah, USA). In this way, a power of 80% would be achieved.

One-way ANOVA was used to compare groups for baseline characteristics and mean BREAST-Q^{TM} scores. Tukey's post hoc test was then used to assess the differences between groups. Normal distribution of the numeric variables was assessed by the Kolmogorov–Smirnov test and Levene's test was used to evaluate homogeneity of variance. Data transformation was conducted to meet the assumptions of the ANOVA (normality and homogeneity of variances). When data did not meet one-way ANOVA's assumptions after data transformations, the Kruskal–Wallis test was used, followed by the Mann–Whitney-U test to determine which groups were different. Bias-corrected and accelerated confidence intervals (1000 repetitions CI

95%) were used to validate results from ANOVA at a significance level of 0.05.(Haukoos and Lewis, 2005, Carpenter and Bithell, 2000) The average income by post code was sourced from the Australian Taxation Office (ATO), www.ato.gov.au, for the financial year between June 2014 and June 2015. The average income for that post code area was then entered against the women's BREAST-Q scores and then pearson correlations were determined by comparing salary with BREAST-Q domain. Statistical calculations were performed using SPSS v22.0 software, (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA) with confidence intervals (CI) represented by lower bound (LB) and upper bound (UB) scores.

3.4 Results

3.4.1 Baseline characteristics and questionnaire response rate.

The baseline characteristics for each group are summarised in Table 1. The number of patients identified as having breast-conserving surgery (without reconstruction) during the recruitment period was 181. Twelve were excluded due to death or progression to total mastectomy. The remaining 169 women who had undergone breast conserving surgery were mailed questionnaires.

In the data set from the Flinders Breast Reconstruction Service, there were 94 women who had answered the BREAST-Q^{TM} following total mastectomy (without reconstruction), and 88 women who had completed the BREAST-Q^{TM} following total mastectomy and breast reconstruction (both mound and nipple areolar complex reconstruction). All patients asked to complete the questionnaire in clinic did so. However, one patient was excluded from each group, based on failure to complete more than 50% of the questions, leaving 93 and 87 women in the respective groups.

Twelve (13.7%) reconstructions were immediate; nine were mixed immediate and delayed (10.3%); and 66 (76%) were delayed. The method of reconstruction was implant-based in 35 (40.2%), latissimus dorsi flap(s) in 31 (35.6%), and free transverse rectus abdominis myocutaneous (TRAM) flap(s) in 21 (24.1%).

One hundred and four returned one or both questionnaires (98 BREAST-Q^{TM}'s, 104 adjunct questionnaires, 57.9% BREAST-Q^{TM} response rate). One hundred and twenty-four control participants returned one or both questionnaires (124 BREAST-Q^{TM}'s, 123 adjunct questionnaires, 42% BREAST-Q^{TM} response rate). Of the 222

BREAST-Q^{TM} questionnaires returned, 220 were suitable for inclusion, with two participants excluded for answering less than 50% of the questions. All 227 adjunct questionnaires were completed in full and suitable for analysis

Table 3-1 Patient characteristics

Treatment Group		**Controls** **n = 123**	**Breast-Conserving Surgery** **n= 104**	**Total mastectomy without reconstruction** **n= 93**	**Total mastectomy with reconstruction** **n=87**	**p Value (Significance <0.5)**
Median Age (Range)		46 (20-87)	62 (38-94)	55 (31-80)	54 (29-70)	0.075
Tumour Size in mm† (Range)		n/a	22.0 (2-67)	23.1 (6-34)	26.8 (4-41)	0.134
Tumour Grade - No. of patients (Proportion of treatment group as a %)[#†]	Grade 1	n/a	19 (18.2%)	6 (4%)	6 (7.3%)	
	Grade 2	n/a	48 (46.1%)	31 (38.2%)	33 (40.2%)	
	Grade 3	n/a	36 (34.6%)	44 (54.3%)	43 (52.4%)	
Lymph Node Involvement - No. of patients with positive nodes† (Proportion of treatment group as a %)		n/a	21 (20.5%)	37 (46.2%)	22 (27.8%)	

Radiotherapy status (Proportion of treatment group as a %)	Irradiated	n/a	65 (62.5%)	55 (67.9%)	37 (45.1%)	
	Not Irradiated		39 (37.5%)	26 (32.1%)	45 (54.9%)	
Time Since Cancer Resection Surgery Mean in months (Range)		n/a	28.9 (12-58)	16.8 (14-68)	48.0 (3- 114)	<0.010*

[#]In one breast conserving surgery patient tumour grade was not specified.

*Difference between groups significant for one-way ANOVA at <0.01, Tukey post- hoc analysis showed: Recon vs. Breast Conserving Surgery (p=0.001) & Recon vs. Total Mastectomy (p=0.001).

† 12 participants in the total mastectomy without reconstruction group and 5 in the total mastectomy with reconstruction group did not have histopathology reports available. Data is based on remaining participant's reports.

3.4.2 BREAST-Q Scores.

Scores from the BREAST-Q questionnaires are shown in Table 2. In the domain of "Satisfaction with Breasts"(Figure 1.), the total mastectomy without reconstruction group had the lowest scores compared to the other groups. The total mastectomy with reconstruction group had the highest scores, with there being little difference between different timings of reconstruction (immediate group mean = 73.75 (95% C.I., 88.02, 59.48), mixed group mean = 82.11 (95% C.I. 92.48, 71.74), delayed group mean = 75.63 (95% C.I. 79.75, 71.95) (Figure 2.). There was no significant difference for the "Satisfaction with Breast" scores between patients who had undergone breast conservation vs. controls. Women who had undergone breast conservation without reconstruction were significantly more satisfied with their breasts than patients who had undergone total mastectomy without reconstruction, but significantly less satisfied than patients who had undergone reconstruction after total mastectomy.

BREAST-Q^{TM} scores for the domain of "Psychosocial Well-Being" were similar for controls vs. women who had undergone breast conservation (Figure 3). They were also similar to women who had undergone total mastectomy with or without reconstruction.

"Physical Well-being Chest" scores showed that women who underwent breast conservation scored significantly lower (i.e. had more pain and discomfort) than women in the control group (Figure 4). Women who had undergone breast conservation also had significantly worse "Physical Well-being Chest" scores than women who had undergone a total mastectomy with or without reconstruction.

The domain of “Sexual Well-being” had fewer responses than the other three domains of the BREAST-Q^{TM} (76.2% following breast conservation, 91.7% controls, 90.3% total mastectomy without reconstruction, 90.8% total mastectomy without reconstruction group participants) (Figure 5.). The scores for patients who had undergone breast conservation were not significantly different from those reported by controls. “Sexual Well-Being” scores were significantly higher in women who had undergone breast conservation compared to women who had undergone a total mastectomy without reconstruction and lower than in women who had undergone a total mastectomy and a breast reconstruction.

Table 3-2 BREAST-Q™ Scores in different domains versus treatment group and comparison between scores of different treatment groups.

All scores are shown as mean (95% confidence interval, upper bound, lower bound).

	Treatment Group	BREAST-Q™ Score	Control	Breast Conserving Surgery (BCS)	Total mastectomy without reconstruction (Mx alone)	Total mastectomy with reconstruction (Mx&recon)
Satisfaction with Breasts	Control (n=123)	60.45 (63.79, 56.87)	n/a	p= 0.81	p < 0.001	p < 0.001
	BCS (n=97)	61.80 (68.32, 57.58)	p = 0.81	n/a	p < 0.001	p < 0.001
	Mx alone (n=93)	38.26 (41.46, 35.47)	p < 0.001	p < 0.001	n/a	p < 0.001
	Mx & recon(n=76)	77.20 (80.72, 73.60)	p < 0.001	p < 0.001	p < 0.001	n/a
Psychosocial Well-being	Control (n=122)	68.54 (72.35, 65.79)	n/a	p=0.970	p = 0.892	p > 0.5*
	BCS (n=97)	68.71 (73.76, 64.65)	p = 0.970	n/a	p = 0.887	p > 0.5*
	Mx alone (n=92)	67.20 (70.4, 63.99)	p = 0.892	p = 0.887	n/a	p > 0.5*
	Mx& recon (n=87)	80.51 (84.59, 76.42)	p > 0.5*	p > 0.5*	p > 0.5*	n/a

	Treatment Group	BREAST-Q™ Score	Control	Breast Conserving Surgery (BCS)	Total mastectomy without reconstruction (Mx alone)	Total mastectomy with reconstruction (Mx&recon)
Physical Well-being Chest	Control (n=118)	82.31 (84.75, 79.86)	n/a	p < 0.001	p = 0.772	p = 0.562
	BCS (n=96)	71.50 (74.84, 68.16)	p < 0.001	n/a	p < 0.001	p < 0.001
	Mx alone (n=93)	80.14 (84.15, 76.13)	p = 0.772	p < 0.001	n/a	p = 0.986
	Mx& recon (n=86)	79.30 (82.69, 75.91)	p = 0.562	p < 0.001	p = 0.986	n/a
Sexual Well-being	Control (n=111)	53.26 (56.70, 49.82)	n/a	p = 0.107	p = 0.001	p < 0.001
	BCS (n=74)	45.84 (52.01, 39.67)	p = 0.107	n/a	P = 0.001	p < 0.001
	Mx alone (n=84)	32.19 (36.92, 27.46)	p < 0.001	p = 0.001	n/a	p < 0.001
	Mx& recon (n=79)	66.06 (70.82,61.31)	p < 0.001	p < 0.001	p < 0.001	n/a

*Did not meet the assumptions of ANOVA, failed Levene's Test.

Figure 3-1 Satisfaction with Breast scores versus main treatment groups.

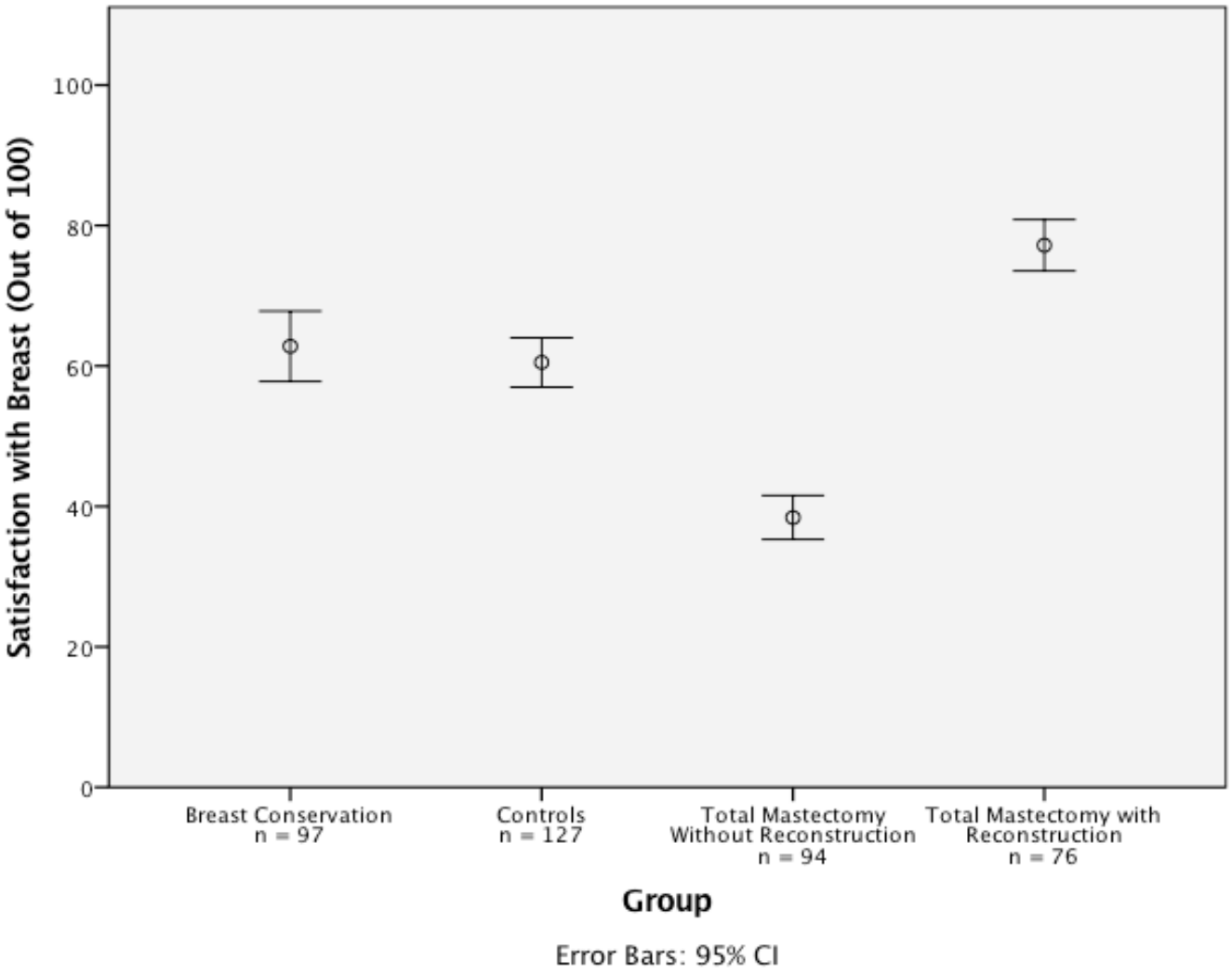


Figure 3-2 Satisfaction with Breast scores versus treatment subgroups.

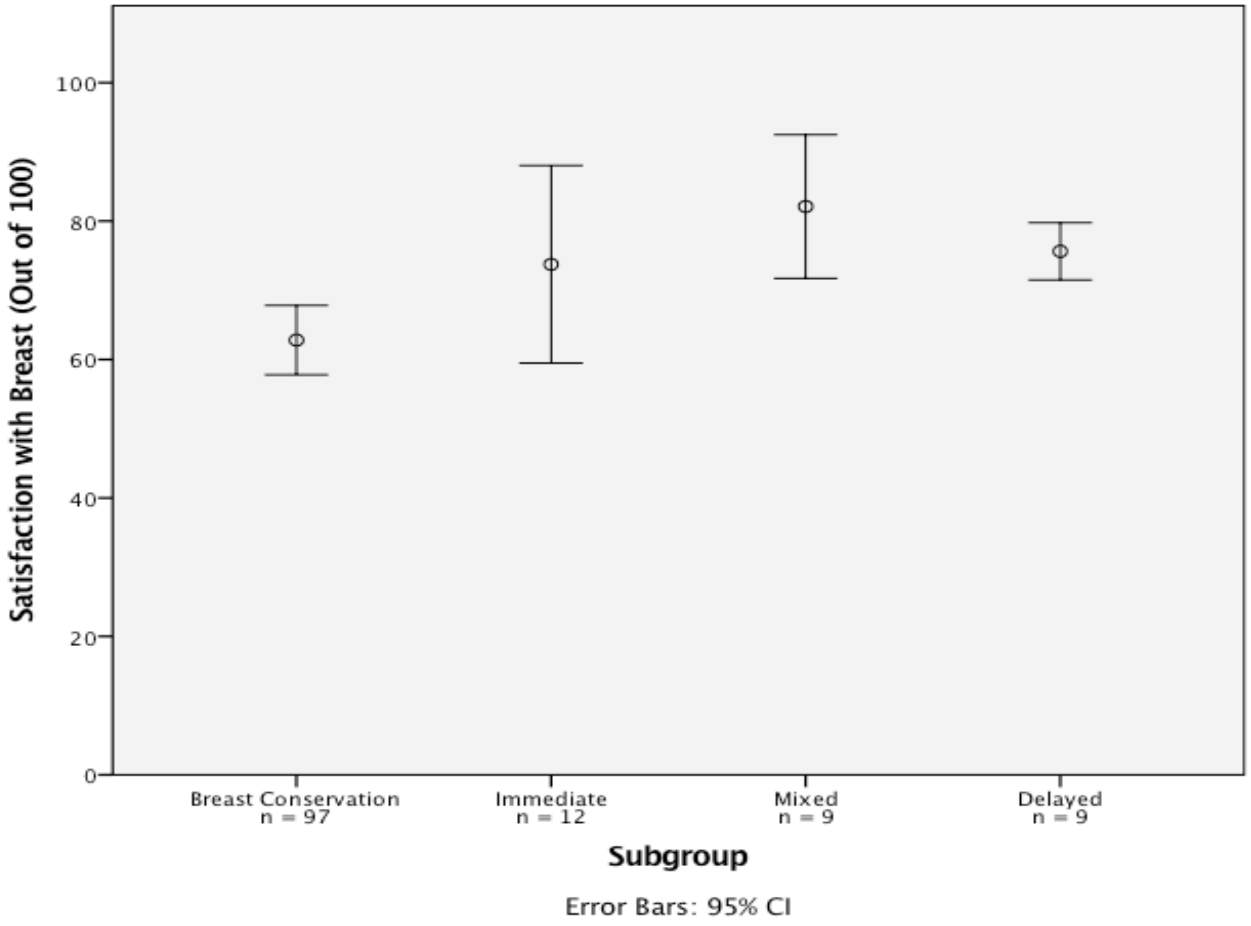


Figure 3-3 Psychosocial Well-being versus main treatment groups.

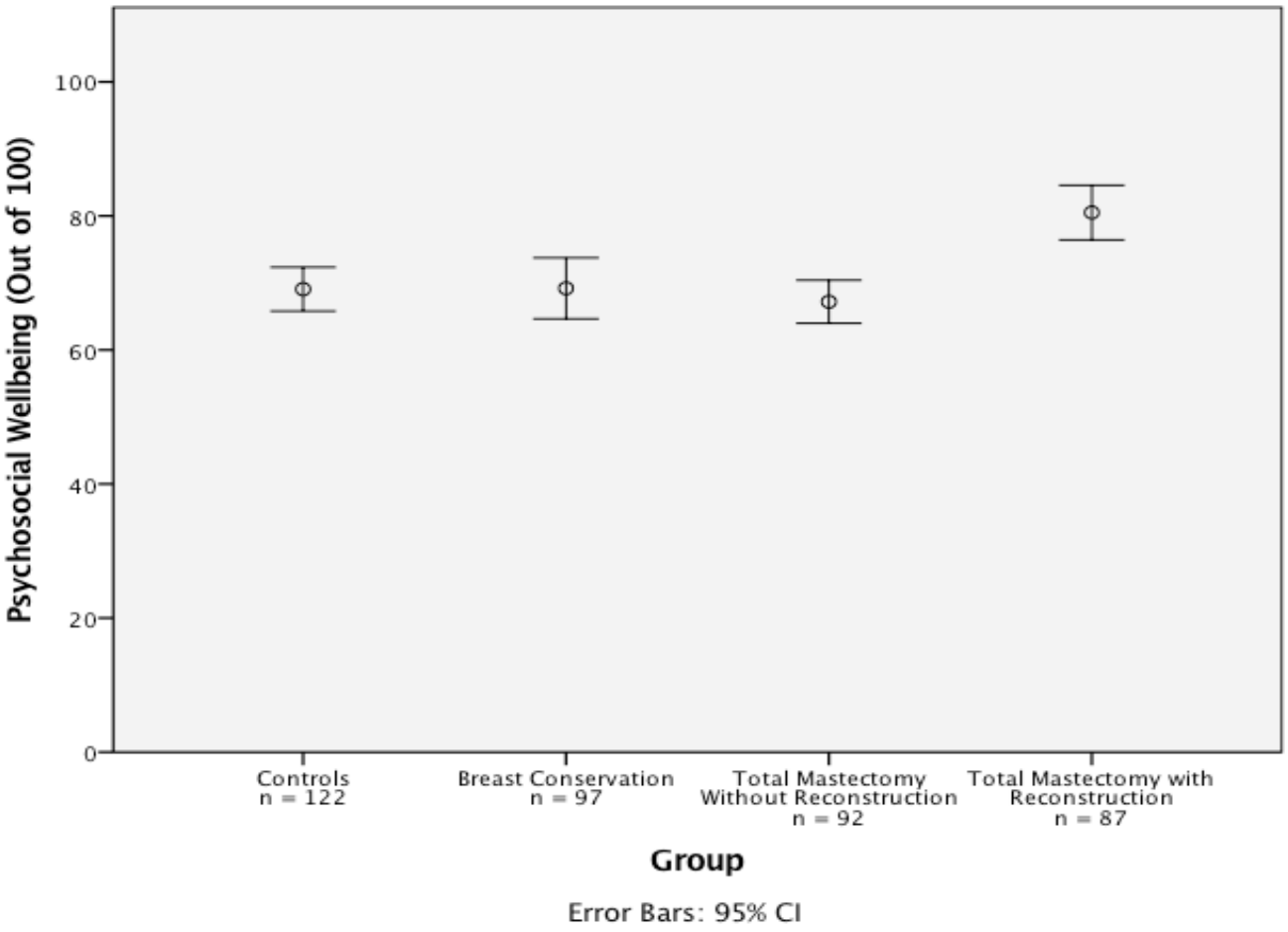


Figure 3-4 Physical Well-being Chest scores versus main treatment groups.

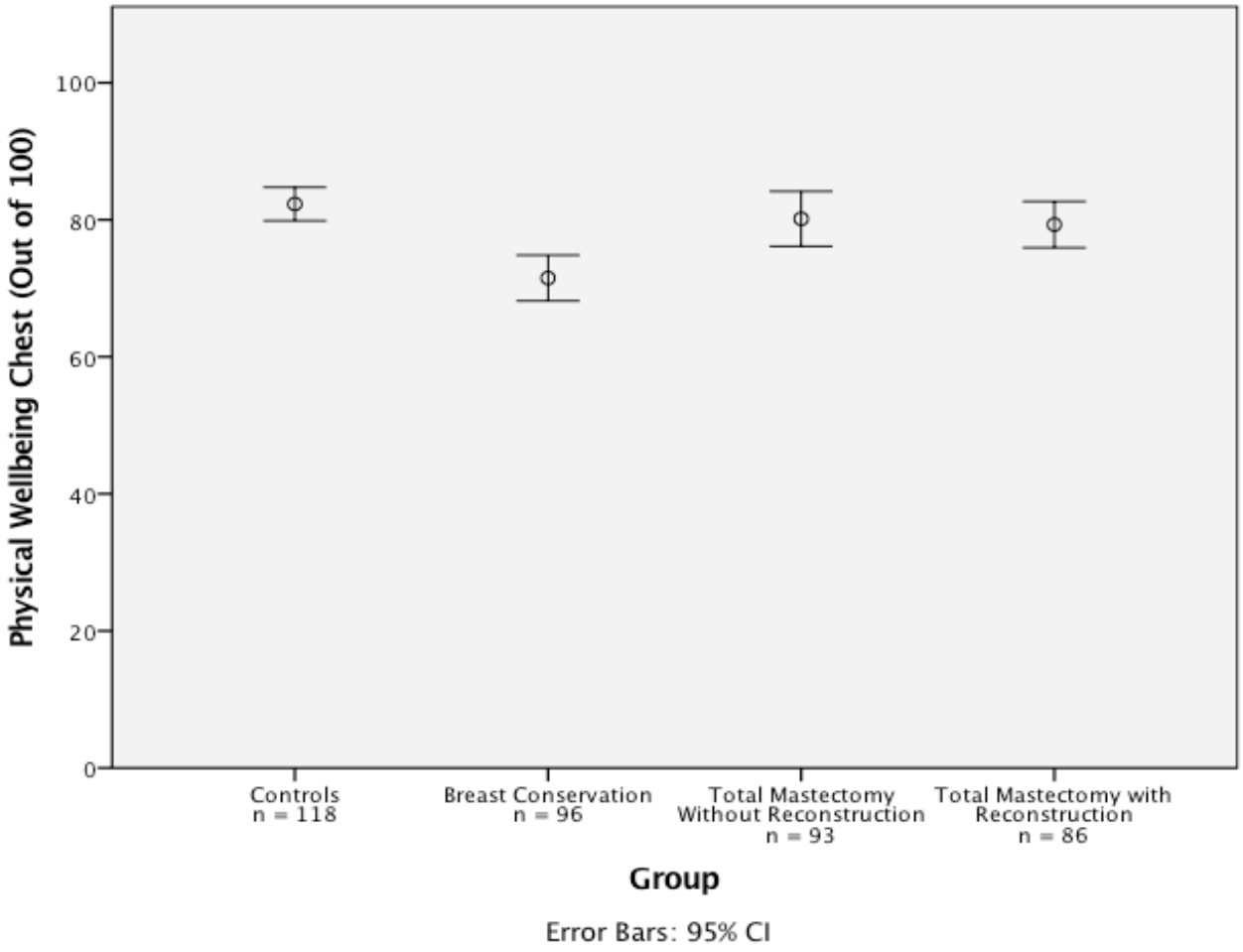
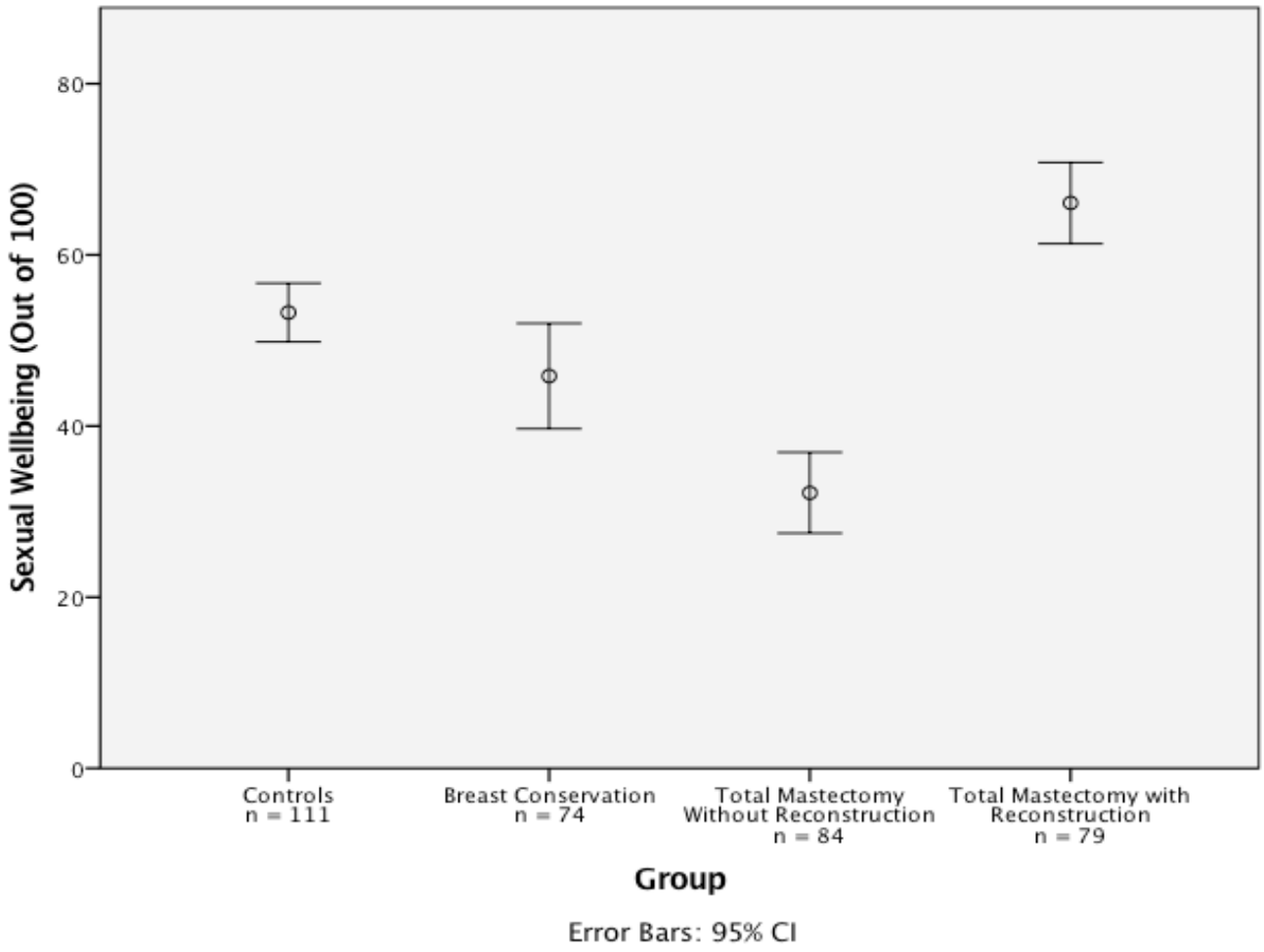


Figure 3-5 Sexual Well-being scores versus main treatment groups.



3.4.3 Missing data for groups are shown below in Table 3. Missing data was excluded from final statistical analysis.

Table 3-3 Data shown are number of patients who failed to complete a section of the BREAST-Q^{TM} relating to any of the four domains of interest. The BREAST-Q^{TM} is designed to function so that if one section is incomplete, the other sections are still valid and can be used for analysis.

Missing Data	Satisfaction with Breast	Psychosocial Well-being	Physical Well-being Chest	Sexual Well-being
Controls	0	1	5	12
Breast-Conserving Surgery	7	7	8	30
Total mastectomy without reconstruction	0	1	0	9
Total mastectomy with reconstruction	11	0	1	8

3.4.4 Breast conserving surgery BREAST-Q domain comparisons, age and income correlations

There was a positive correlation between “Satisfaction with breast” and age in women who had undergone breast conserving surgery, with “Satisfaction with breast” increasing with increasing age ($r^2 = 0.47$, p=0.01). This was true also for “Psychosocial wellbeing” ($r^2 = 0.37$, $p = 0.001$) and “Physical wellbeing chest” ($r^2 = 0.28$, $p = 0.005$).

For women who had undergone breast conserving surgery there was no correlation between “Satisfaction with breast” vs income, “Physical wellbeing chest” vs income, “Psychosocial wellbeing” vs income or “Physical wellbeing chest” and income. However, there was a correlation between “Sexual wellbeing” and income ($r^2 = 0.235$, $p < 0.014$) with higher income earners experiencing higher sexual wellbeing scores.

When comparing BREAST-Q domains from women who had undergone breast conserving surgery there were positive correlations between “Satisfaction with breast” vs “Psychosocial wellbeing” and “Satisfaction with breast” vs “Sexual wellbeing” ($r^2 = 0.629$, $p<0.001$). With regard to “Physical wellbeing chest”, as previously mentioned, a higher score denotes less pain and discomfort than a lower score. As women who had breast conserving surgery experienced less pain and discomfort in their chest area compared to the other groups, so too did they experience more satisfaction with breast, psychosocial and sexual wellbeing

Figure 3-6 Breast Conserving Surgery: Satisfaction with breast vs physical wellbeing chest

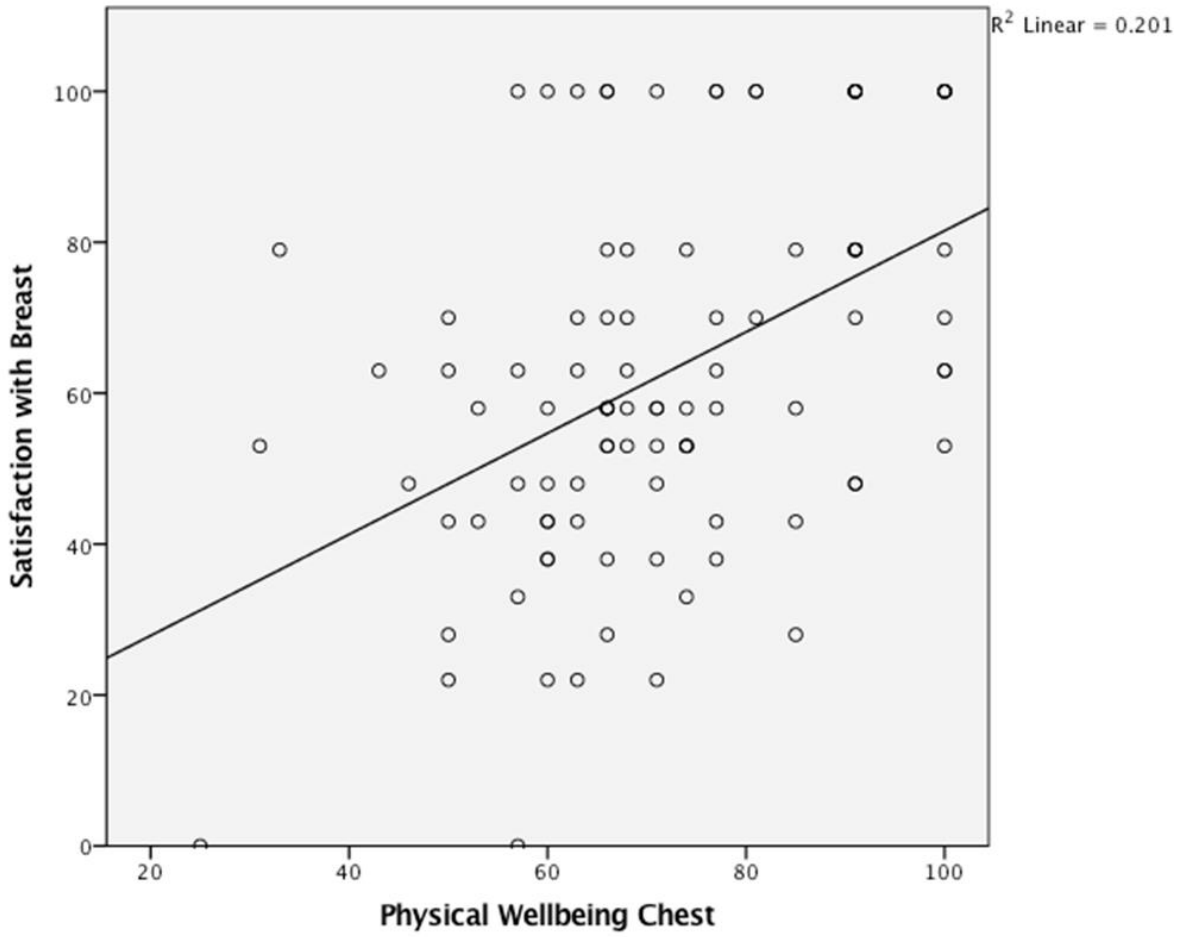


Figure 3- 7. Breast Conserving Surgery: physical wellbeing chest vs psychosocial wellbeing

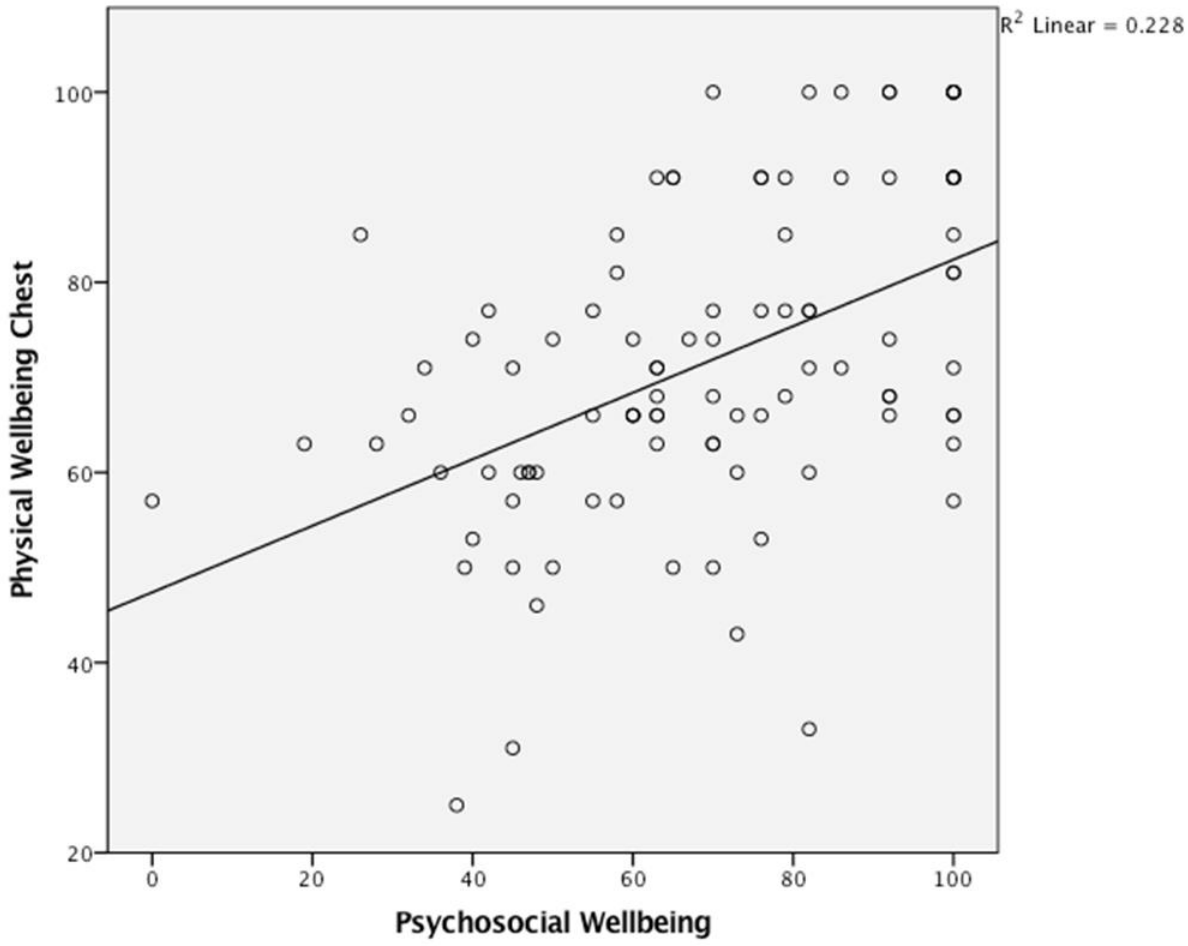
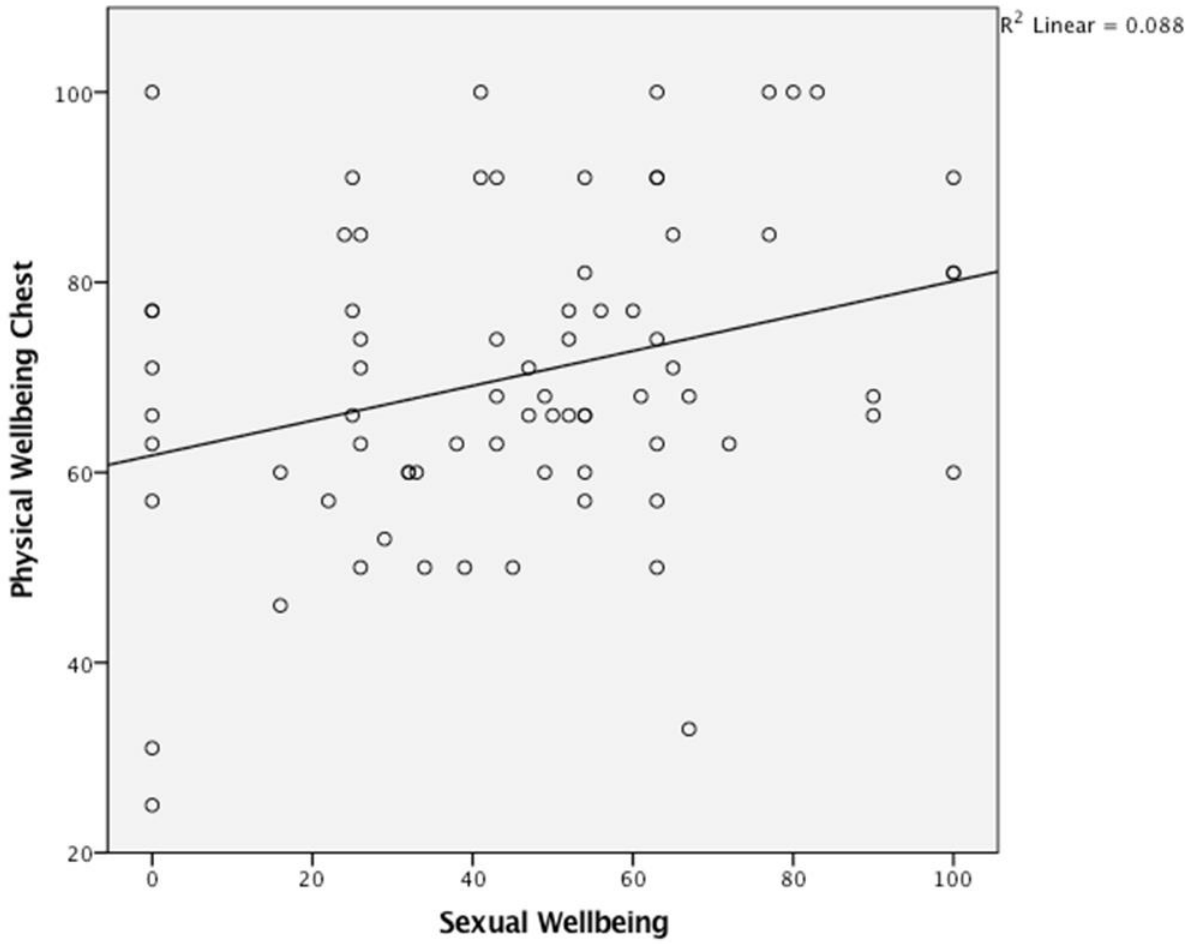


Figure 3-8. Breast Conserving Surgery: physical wellbeing chest vs sexual wellbeing



3.4.5 Radiotherapy and subgroup analysis

The effect of radiotherapy on the quality of life was investigated with subgroup analysis. Table 4 demonstrates the differences between the breast conserving surgery group with and without radiotherapy as well as the total mastectomy group with reconstruction.

Table 3-4 Difference between breast conserving surgery (BCS) versus total mastectomy with and without radiotherapy

	Satisfaction with BREAST			Psychosocial Well-being			Physical Well-being Chest			Sexual Well-being		
Treatment Group	Partial Mastectomy: No Radiotherapy	Total Mastectomy (with Reconstruction)	Partial Mastectomy & Radiotherapy	Partial Mastectomy: No Radiotherapy	Total Mastectomy (with Reconstruction)	Partial Mastectomy & Radiotherapy	Partial Mastectomy: No Radiotherapy	Total Mastectomy (with Reconstruction)	Partial Mastectomy & Radiotherapy	Partial Mastectomy: No Radiotherapy	Total Mastectomy (with Reconstruction)	Partial Mastectomy & Radiotherapy
Mean BREAST Q Score (Confidence Interval)	**61.5 (55.1- 67.4)**	**76.5 (73- 80.3)**	**60.8 (51.8- 69.3)**	**74.9 (66.5- 88.9)**	**80.2 (76.4- 84.2)**	**67.1 (61.4- 72.4)**	**74.9 (68- 81.5)**	**74.9 (68- 81.5)**	**69.8 (65.9- 73.7)**	**47.1 (34.48- 60.84)**	**65.44 (60.79 – 69.6)**	**45.8 (39.2- 52.7)**
	↘	↙ ↘	↙	↘	↙ ↘	↙	↘	↙ ↘	↙	↘	↙ ↘	↙
Significance between Groups	**p=0.905**	**p=0.004**		**p=0.109**	**p=0.001**		**p=0.156**	**p=0.012**		**p=0.847**	**p= 0.00018**	

3.4.6 Adjunct Questions.

Twelve controls reported breast asymmetry (10%), and these individuals all indicated they would consider surgical remediation. Seventy-four (75.25%) women who had undergone breast conserving surgery perceived themselves as having breast asymmetry with fifteen (15.5%) indicating this to be a problem for which they would consider surgery. There was no difference between BREAST-Q^{TM} scores of those who had received radiotherapy versus those who had not (data shown 3.3.2).

3.5 Discussion

In this study, women who had undergone breast-conserving surgery for breast cancer had similar quality-of-life outcomes to controls for satisfaction with their breasts, psychosocial well-being and sexual well-being, confirming good outcomes following breast conservation, and comparable quality-of-life outcomes to women who have not had breast cancer. The desire for surgery to improve breast asymmetry was only slightly higher in the breast conservation group compared to controls, further confirming good outcomes following breast conservation (Fosh et al., 2014). The rate of perceptible asymmetry after breast-conserving surgery in our study was similar to findings reported by Durand et al. who identified fair to poor cosmetic results in 23% of women following breast conservation.(Gorczyca et al., 2007)

In our current study, women who had undergone total mastectomy with breast reconstruction also had good outcomes in terms of satisfaction with their breasts, psychosocial well-being and sexual well-being, and these outcomes were at least as good as those reported following breast conservation. Breast reconstruction after mastectomy has long been known to improve patient satisfaction and quality of life. Dean et al. have previously demonstrated a benefit from immediate breast reconstruction by reducing the incidence of post-operative depression, compared to women undergoing delayed breast reconstruction.(Dean et al., 1983) Zhong et al. also demonstrated improvement between pre-operative and post-operative measurements of satisfaction with breast, psychosocial and sexual well-being in patients who had undergone transverse rectus myocutaneous and deep inferior epigastric perforator flap reconstructions.(Zhong et al., 2012)

Breast reconstruction is an ongoing area of interest as mastectomy rates are

increasing globally.(2011, Zhong et al., 2012, Eltahir Y, 2014, Elder et al., 2005, Mahmood et al., 2013, King et al., 2011, Yao et al., 2010). Recent studies from the USA have demonstrated increasing rates of total mastectomy both for the affected breast and as prophylactic and contralateral procedures in the context of high cancer risk.(Garcia-Etienne et al., 2012, Mahmood et al., 2013, King et al., 2011, Yao et al., 2010) Evaluation of quality of life following breast conservation vs. total mastectomy and reconstruction is very relevant to current practice in view of the increasing rates of total mastectomy for breast cancer. Only one other study has investigated this issue using a quality-of-life questionnaire. Al-Ghazal et al. reported a retrospective series identifying greater satisfaction and psychosocial well-being in patients following breast conservation vs. mastectomy and reconstruction.(Al-Ghazal et al., 2000) However, their study used generic, rather than breast-specific, outcome measures, including the Hospital Anxiety Depression Scale, the Body Image Scale and the Rosenberg Self-Esteem Scale, and this choice might explain differences with our study, which used a breast-specific quality-of-life measure.

Currently, only a small minority of women who have had breast conservation undergo reconstructive surgery. Our study suggests there might be a cohort of patients who consider their breast asymmetry to be problematic and would consider surgical remediation. Contour deformities following breast conservation, particularly in those that undergo adjuvant radiotherapy, pose a complex challenge, and limited options include breast reduction or importing tissue with local perforator flaps (Kronowitz et al., 2006). If the pain and discomfort women are experiencing is related to the radiotherapy then autologous fat grafting may assist in reversing the effects of radiotherapy damage to native breast tissue (Rigotti G., 2005, Sarfati et al., 2011, Ribuffo et al., 2013, Maione et al., 2014). When observing the relationships in

the correlations between physical wellbeing chest and the other BREAST-Q domain, if autologous fat grafting reversed the effects of radiotherapy, this may, in turn, improve satisfaction with breast, psychosocial wellbeing and sexual wellbeing. The role of autologous fat grafting for breast defect correction is an area warranting investigation in this context.

A significant proportion of the patients in our study underwent delayed, rather than immediate, reconstruction. It could be argued that mean quality-of-life scores may have been higher in patients who had adjusted to the loss of their breast following total mastectomy before seeking defect correction. If true, then such scores might not be replicated with implementation of a higher rate of mastectomy with immediate reconstruction. A potential issue with generalising our findings is that the women in the post-mastectomy group were all attending a breast reconstruction clinic for consideration for reconstructive surgery. Hence, they self-selected for possible reconstruction, and therefore might not be representative of the full spectrum of post-mastectomy patients. Kelsall et al. reviewed 286 women who had undergone breast conserving surgery and 281 women who had undergone total mastectomy and immediate breast reconstruction using the body image scale (BIS) and an institute specific patient reported outcome measure (Smith et al., 2006). They found that in self-rated breast appearance, return to work and function women who had undergone breast conserving surgery had significantly higher scores compared to women who had undergone total mastectomy and immediate breast reconstruction. Although these findings are interesting, when comparing breast conserving surgery to total mastectomy and breast reconstruction the nature of these procedures are quite different. Whether the reconstruction is with the insertion of an expander or via autologous flap reconstruction there are going to be differences in operative time,

length of hospital stay, length of incision scars and associated morbidity compared with breast conserving surgery. Insertion of a breast expanders involves the creation of a subpectoral pocket beneath the pectoralis major muscle. In autologous flap reconstruction using a DIEP flap, ribs in the chest need to be removed to allow access for microsurgical anastomosis of arteries and veins. Despite obvious technical differences when assessing breast appearance breast conserving surgery maintains native breast tissue which will have difference aesthetics to a total mastectomy and autologous flap reconstruction. Return to work with breast conserving surgery is likely to result in less morbidity and women are likely to be less 'functional' after autologous flap reconstruction owing to longer hospital stay. Unfortunately the Hopwood Body Image Scale (BIS) is heavily balanced towards breast aesthetics only and does not have long term morbidity questions that may have revealed the long-term sequelae of radiotherapy related breast fibrosis (Fisher et al., 2013).

We were surprised that the women in our study who had undergone breast conserving surgery without reconstruction reported more pain and discomfort in the chest area than both controls and the women who had undergone total mastectomy with and without reconstruction. The radiotherapy sub-group analysis is relevant because breast conserving surgery and radiotherapy has been the gold standard for treatment of early breast cancer for several decades (Fosh et al., 2014). This subgroup analysis suggests that total mastectomy and reconstruction provides better patient reported outcomes than breast conserving surgery and radiotherapy. However, this study was not designed to investigate the factor of radiotherapy a priori and these findings cannot be taken as definitive. A larger study, specifically looking at the effect of radiotherapy on the breast may be warranted.

Another possible weakness in our study is that the BREAST-Q^{TM} version we used was not specific for breast-conserving surgery. The BREAST-Q^{TM} Breast Conserving Therapy module was still in development as we were conducting the current study and was not available for our use. However, all individuals in our study answered the same questionnaire, and this made it easier to compare groups than if we applied a different questionnaire to different cohorts. Further research is underway to definitively establish what threshold of difference between BREAST-Q^{TM} results is clinically important (Pusic, A., personal communication). Once these "minimal important difference" values are available, this will make research with this outcome measure more powerful.

Unfortunately, there were no women who had undergone breast cancer surgery and autologous fat grafting for breast reconstruction at the time this study was undertaken. It would have been ideal to include two additional group of women who had undergone autologous fat grafting (post breast conserving surgery and post total mastectomy) to compare with women who had undergone breast conserving surgery, controls, total mastectomy (with and without reconstruction).

When evaluating responses from a postal questionnaire, there is also potential response bias between non-respondents vs. respondents. In a questionnaire addressing psychosocial well-being following breast cancer surgery, women who experienced poor outcomes or had lower psychosocial well-being might not have wanted to explore these feelings by answering a questionnaire. Conversely, during the 'call back' there were patients who explained that they considered themselves cured of cancer and had 'moved on'. It is difficult to ascertain the direction and magnitude of bias, if any, related to the study response rate.

3.6 Conclusion

Fifteen percent of women who have undergone breast conserving surgery would consider surgical remediation. Autologous fat grafting could be an ideal procedure for minor defect correction. Furthermore, this study suggests that women who undergo total mastectomy and breast reconstruction for cancer achieve a good quality of life, and the quality of life outcome was at least as good as that achieved following breast-conserving surgery. Furthermore, breast conservation was associated with more pain and discomfort in the chest area and poorer sexual well-being outcomes. This information suggests that the quality-of-life outcomes in women undergoing total mastectomy and breast reconstruction might actually exceed the expectations of most patients with breast cancer. These outcomes warrant further evaluation in prospective studies set up to specifically address this question. The use of autologous fat grafting in women who have undergone breast conserving surgery and total mastectomy is an area which warrants further investigation and this will be explored in Chapters 4, 5 and 6.

Chapter 4. Autologous fat grafting for whole breast reconstruction*

*A condensed version of this chapter was published: HOWES, B. H., FOSH, B., WATSON, D. I., YIP, J. M., EATON, M., SMALLMAN, A. & DEAN, N. R. 2014. Autologous fat grafting for whole breast reconstruction. Plast Reconstr Surg Glob Open, 2**,** e124.

4.1 Introduction

Breast reconstruction options that are available to women who have undergone mastectomy include implant based and autologous flap methods. Breast conserving surgery involves removal of the tumour and a cuff of normal breast tissue which results in clear margins. Total mastectomy involves removal of all breast tissue, nipple areolar complex and overlying tissue. There are few reported cases on the use of autologous fat grafting for whole breast reconstruction (Bircoll M, 1987, Serra-Renom et al., 2011, Babovic, 2010, Panettiere et al., 2009).

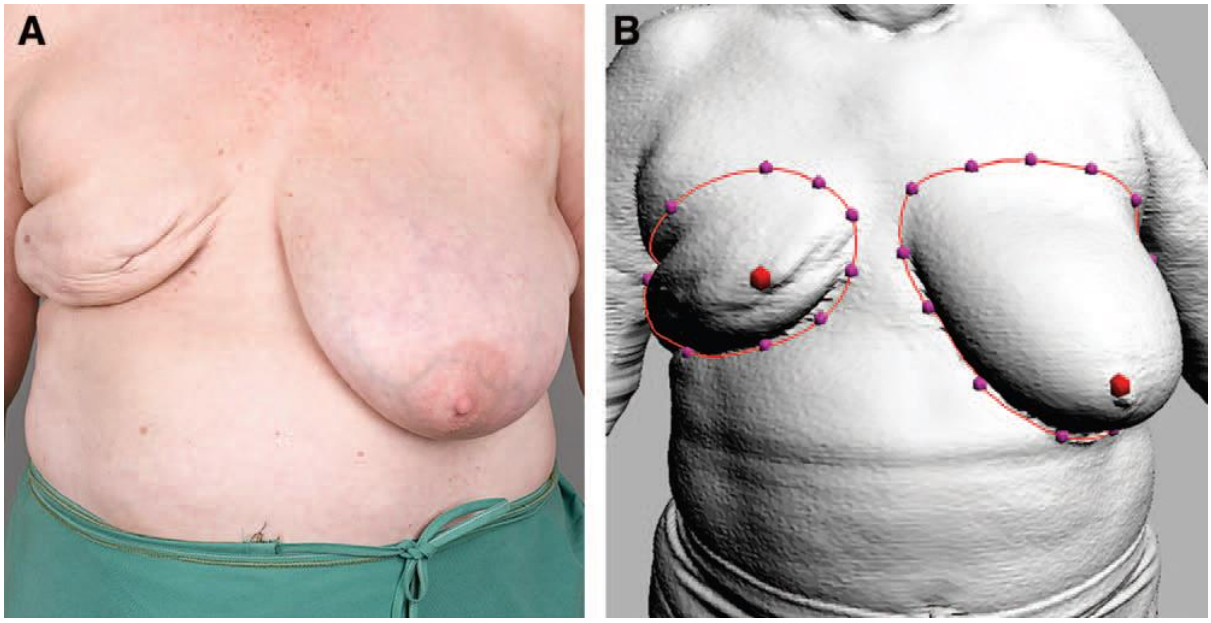
The purpose of this case study was to evaluate the viability of reconstruction of the breast by autologous fat grafting alone, in the context of rotation flap approach (RoFA) mastectomy(Dean N.R, 2013). The hypothesis was that there would be minimal loss of autologous fat volume in the 12 months following surgery.

4.2 Case Study

The patient was a 63-year-old woman who presented with screen-detected multicentric cancer of the right breast. She underwent RoFA mastectomy and sentinel lymph node biopsy. Histopathology showed 2 cancers in separate quadrants of the breast, 17 and 11 mm in size, both of low nuclear grade, and neither showing peritumoural vascular invasion. Lymphoscintigraphy for sentinel node biopsy was obtained by peritumoural injection around the larger of the 2 cancers, and the sentinel and 2 adjacent axillary lymph nodes were

clear of metastatic disease. Although the tumour was hormone sensitive, the patient opted not to have adjuvant hormonal treatment. With a good prognostic outcome, low risk of cancer recurrence, she was an ideal candidate for autologous fat grafting. At the time of presentation to the plastic surgeon for consideration of reconstruction, the patient had a large ptotic breast on the left and the skin envelope subsequent to the RoFA mastectomy on the right was capacious and supple (Figure. 1).

Figure. 1. Preoperative clinical photograph of patient (A). 3D laser scan image with measured volumes (B). 3D breast volume measurement: left breast, 1469 ml; right breast, 251 ml.



The patient was keen to have the left breast reduced in size and was interested in reconstructive options for the right side, but not enthusiastic about either flap reconstructions or prosthetic implants due to perceived morbidity of these procedures. The concept of autologous fat grafting was explained to the patient, and she was warned that the outcome was not predictable and specifically that she was likely to experience some fat graft loss. She was willing to accept this outcome. Eleven months after mastectomy, she underwent right breast reconstruction with autologous fat grafting alone. In the same operation, she underwent a Wise pattern inferior pedicle left breast reduction, with the volume resected being guided by a preoperative volumetric assessment by 3D laser scan. These procedures were uncomplicated, and she was discharged from hospital on the first postoperative day. She had reconstruction of the nipple-areolar shape two months following mound reconstruction (Figure. 2) and had tattooing of the nipple-areolar reconstruction a further 3 months later (Figure. 3).

Figure. 2. Postoperative (3 months) clinical photograph of patient (A). 3D laser scan image with measured volumes (B). 3D breast volume measurement: left breast, 583 ml; right breast, 635 ml.

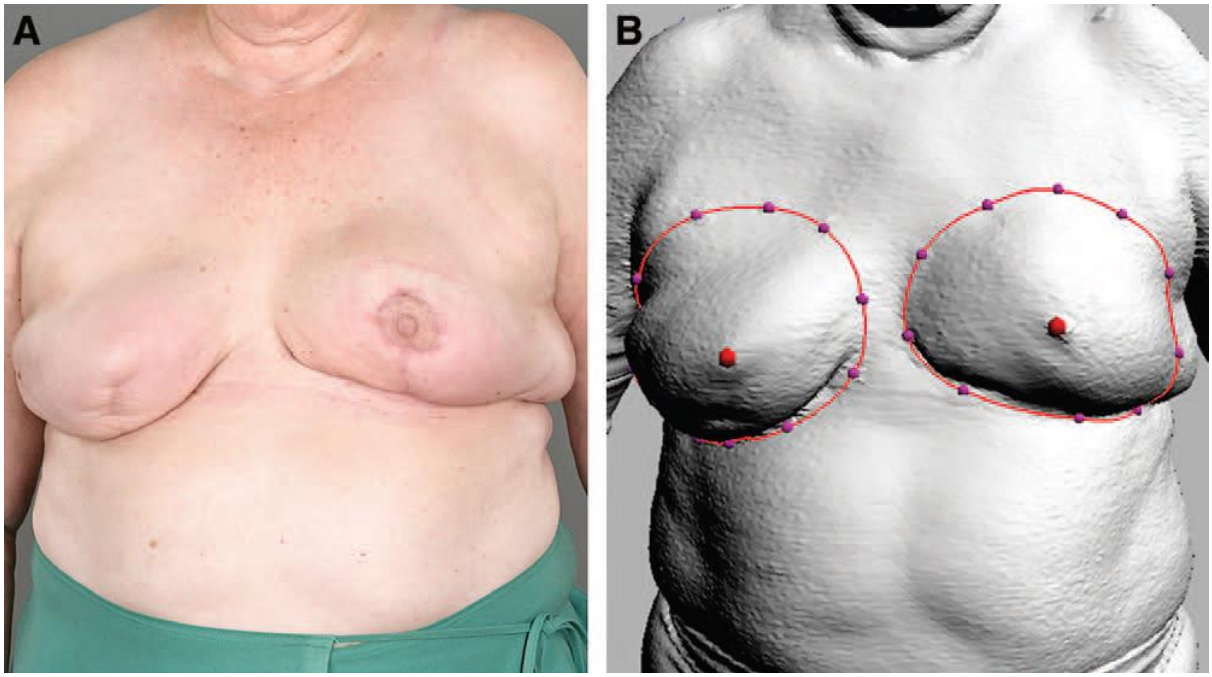
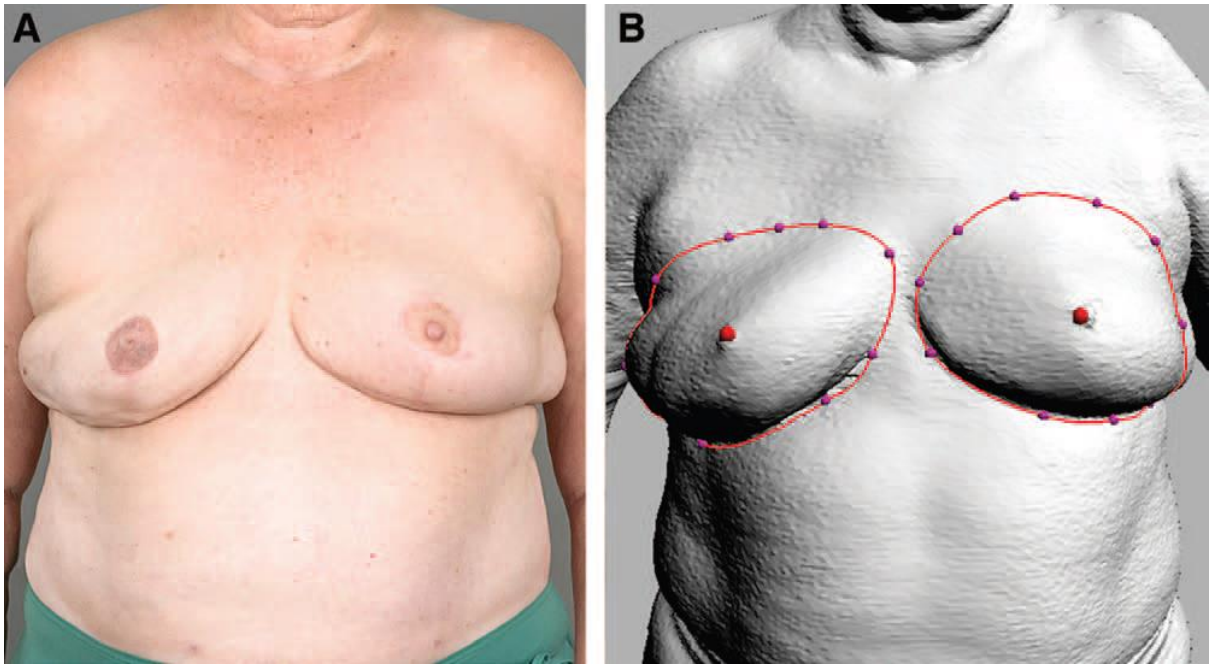


Figure. 3. Postoperative (6 months) clinical photograph of patient (A). 3D laser scan image with measured volumes (B). 3D breast volume measurement: left breast, 634 ml; right breast, 571 ml.



4.3 Methods

The BREAST-Q questionnaire was completed preoperatively and was repeated at 3, 6, and 12 months postoperatively. The raw questionnaire data were converted using the Q-Score program found at the developer's Web site (https://webcore.mskcc.org/breastq/). This provided summary scores for each BREAST-Q scale, ranging from 0 to 100 in 6 "domains" or areas of interest. The 3 "satisfaction" domains were satisfaction with breasts, satisfaction with outcome, and satisfaction with process of care.

The 3 "well-being" domains were physical, psychosocial, and sexual well-being. Three-dimensional (3D) laser scans were performed using a Cyberware whole-body laser scanner and the Cyslice software (Headus, Australia) to measure the volume of the breasts. This was done using the protocol previously described in the validation study by Yip et. al.(Yip J.M, 2012) Image capture was performed using Cyscan (Cyberware), image analysis was performed using Cyslice (Headus) (metamorphosis), and statistical analysis was performed using Stata version 11.0 (StataCorp, College Station, TX). The patient was scanned preoperatively and at 3, 6, and 12 months postoperatively. Operatively, the patient's first procedure was the RoFA mastectomy as previously described by Dean et. al.(Dean N.R, 2013) This technique involves a vertical incision from the areola, dropped medial to the breast axis, down toward the inframammary fold. The incision extends along the inframammary fold laterally to the anterior axillary line. The rotation flap that is created allows access for mastectomy, recruits skin to the apex of the breast, and prevents scarring in the medial half of the chest. Her second procedure was

reconstruction of the whole breast with fat grafting and simultaneous left breast reduction using a Wise pattern inferior pedicle technique. One thousand millilitres of aspirate was harvested from the abdomen using the Lipivage System. Four hundred milliliters of filtered fat was then injected in layers using a vertical and horizontal fanning technique, creating tunnels in several directions within the mastectomy skin envelope. Injection of fat included the pectoral fascia and the pectoralis major muscle. The amount of tissue removed in the left breast reduction was 980 g.

4.4 Results

Preoperative BREAST-Q scores indicated that the patient had good physical well-being but was unsatisfied with her breast area and had low sexual well-being (Table 1). In particular, she was very dissatisfied with wearing fitted clothes and how she looked naked in the mirror. The preoperative difference in breast size is shown in Figure 1. Postoperatively, the wounds healed without complication, and by 3 months, the mastectomy scar had become softer. The reconstructed breast was soft and nontender. The patient reported that she had lost 6 kg in the 6 months after surgery, and this was confirmed using scales and comparing her weight preoperatively. During the period between 6 and 12 months, she had lost a further 3.5 kg. At 3, 6, and 12 months postoperatively, she was very satisfied with the outcome of surgery (Table 1), and symmetry was objectively found to be better than preoperatively (Figures. 1–4). The volumes of the 3D laser scans taken preoperatively and at 3, 6, and 12 months postoperatively are shown beside her 2D clinical photography in Figures 1–4.

Figure. 4. Postoperative (12 mo) clinical photograph of patient (A). 3D laser scan image with measured volumes (B). 3D breast volume measurement: left breast, 521 ml; right breast, 541 ml.

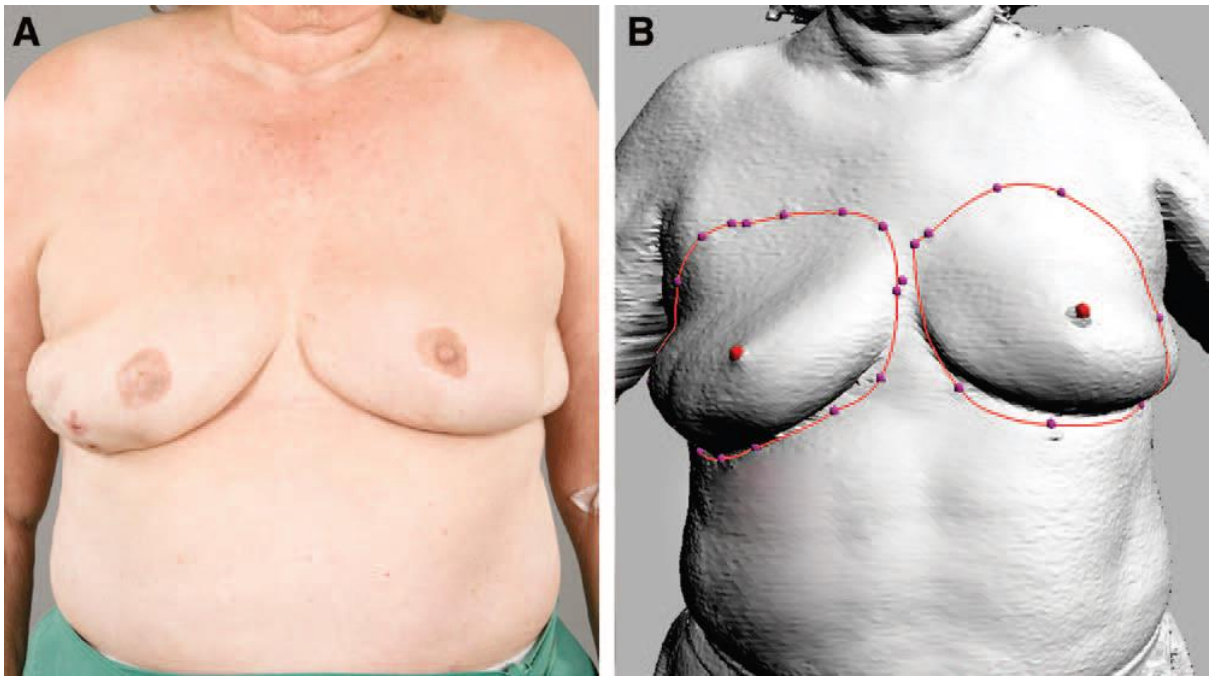


Table 1. Quality of Life Scales with BREAST-Q scores preoperatively, 3, 6 and 12 months post operatively.

	BREAST Q Score			
Quality of Life Scale	Pre-operative	Post-Operative (3 mo)	Post-Operative (6 mo)	Post-Operative (12 mo)
Satisfaction with breast	38	85	91	100
Satisfaction with outcome	61	75	100	100
Psychosocial wellbeing	63	79	100	100
Physical Well-being	85	77	100	90
Sexual Well-being	32	88	63	79

4.5 Discussion

Autologous fat grafting for breast reconstruction is gaining popularity. The most encouraging article by Delay et. al.(Delay et al., 2009a) reported on the technique, results, and indications of fat injection on 880 procedures, spanning 10 years. Rigotti's group (Rigotti G., 2010) followed-up 137 radical mastectomy patients who underwent fat grafting over a 7.6-year period. He did not find an increased risk of cancer compared with the non-treated group.

These results helped to dispel earlier concerns about the oncological risk of fat grafting. Earlier reports on fat grafting suggested that nodule formation and calcifications would interfere with breast cancer screening.(Rosing J H, 2011) In turn, concerns were raised regarding interpretation of mammographic findings leading to a higher false-positive cancer diagnosis. Studies since have found no evidence of fat grafting causing interference with breast cancer screening.(Parrish, 2010, Claro Jr, 2012, Carvajal J., 2008) Calcifications from fat grafting have a benign eggshell appearance.(Rosing J H, 2011) Now with increasing reports of success, and reports of patient satisfaction, surgeons are trying to establish techniques to provide the best results with fat grafting (Delay et al., 2009a, Rigotti G., 2010, Parrish, 2010, Claro Jr, 2012, Carvajal J., 2008, Peer, 2007, Coleman, 2007, Delaporte et al., 2009, Khouri R.K., 2012). Loss of volume can adversely affect patient satisfaction and may require further reconstructive procedures. Previous studies that report graft loss vary greatly in technique, amount lost, and methods in which loss was measured.(Rigotti G., 2010, Parrish, 2010, Claro Jr, 2012, Carvajal J., 2008, Peer, 2007, Coleman, 2007)

The majority of cases reported in the literature discuss fat grafting for breast conserving surgery defects or for aesthetic augmentation purposes. There is little reported on whole breast reconstruction. Panettiere et al (Panettiere et al., 2011) documented success in whole breast reconstruction, but the grafting of fat was carried out in several sessions. Interestingly, in his patient, the mastectomy scar softened after fat grafting, as did the scar in the patient described in this case. Rigotti et al (Rigotti G., 2005) theorized that adipose-derived stem cells can restore ischemic tissue vascularization and organ function by recruitment of endothelial progenitor cells. Using the 3D laser scanner, we were able to accurately measure and compare the maintenance of volume at intervals. The use of 3D laser scanners for assessment of patients undergoing breast reconstruction has been validated in previous studies(Losken A., 2005, Kovacs L, 2005). Yip et al validated the 3D laser scanner (Yip J.M, 2012) that has been assisting our unit in the preoperative preparation phase, the postoperative evaluation of results, and longer term assessment of outcome. The 3D laser scanner has been further validated against magnetic resonance imaging of the breast volume and is discussed further in Chapter 6 of this thesis. It was also used to assess breast volumes in the prospective cohort study to follow in Chapter 5.

The current case demonstrated a volume loss of approximately 25% in a 12-month period. A better outcome when compared with estimated volume losses of 30–40% by Delay et al (Delay et al., 2009a) and 43.5% at 9 months by Beck et al.(Beck M, 2011) Compared with other techniques, the RoFA mastectomy resulted in a large skin envelope, which facilitated reconstruction and aided ptosis, giving a very natural-looking breast. The majority of articles reporting patients' perceptions on outcome do not have quantitative measurement of patient satisfaction. (Delay et al.,

2009a, Rigotti G., 2010, Parrish, 2010, Claro Jr, 2012, Carvajal J., 2008, Peer, 2007, Coleman, 2007, Delaporte et al., 2009, Khouri R.K., 2012) Patient satisfaction was simply registered as good, moderately good, or very good.(Delay et al., 2009a) The BREAST-Q was developed in 2009 to elicit and quantify patient perception of outcomes post augmentation, reduction, and reconstruction(Pusic, 2009). The use of the BREAST-Q pre and post autologous fat grafting demonstrates interval improvement. Our hypothesis that there would be minimal loss of breast volume in the reconstructed side was supported in this case. There was slight interval change with a volume difference of 64 ml between 3 and 6 months, then only 30 ml between 6 and 12 months. Interestingly, the 3D scanner detected increase in volume on the left reduction side despite the patient's weight loss of 6 kg. Superimposing the 3D images, it can be observed that there was a slight difference in the breast border mapping from the body surface markers on the left.

4.6 Conclusion

This case has demonstrated promising results using two new techniques, the RoFA mastectomy and whole breast reconstruction with autologous fat grafting, and confirms the potential for fat grafting as an option for whole breast reconstruction. Autologous fat grafting for breast reconstruction in women who have undergone breast conserving surgery and total mastectomy is an area that warrants further investigation with further prospective studies which have validated quantifiable outcomes measures. This will be explored further in Chapter 5.

Chapter 5. Efficacy of the BRAVA® Device and autologous fat grafting for breast reconstruction in breast-conserving surgery & total mastectomy patients: A prospective pilot study

5.1 Introduction

The scientific principle of growing tissue using isotropic distracting forces has had several medical applications over the last three decades such as limb lengthening and breast reconstruction (Khouri R.K., 2012). The application of tissue expansion with internal expanders was described by Radovan in 1982 for two-stage breast reconstruction (Tepper et al., 2009) whereas the application an external expander device using suction has only been in practice in the last decade. The BRAVA® device has been designed to expand the skin and underlying tissues of the breast using low pressure suction (Khouri R.K., 2012). Initially developed for breast enhancement for cosmetic purposes, its application now includes breast reconstruction for patients post-breast conserving and total mastectomy procedures. Currently, the BRAVA® device is being used in USA, Europe, China, and Australia, in conjunction with autologous fat grafting, as an alternative to traditional reconstructive procedures (Uda et al., 2014, Schlenz and Kaider, 2007). This system is used perioperatively in an attempt to improve fat graft retention rates, because fat reabsorption is the greatest limitation of the fat grafting procedure. This was first demonstrated by Lyndon Peer who grafted fat into the rectus sheath in humans and noted that only 50% of graft had survived at 12 months (Peer, 2007). By expanding the breast skin, the BRAVA® creates a breast envelope into which fat can be grafted. It has also been theorised to improve the vascularity of the recipient site

through mechanical stimulation, creating a ‘fibrovascular scaffold’. One theory of the mechanism that improves vascularity points to a cyclical pattern of use, which was investigated in a pre-clinical mouse model (Rees, 1975). Reportedly, cyclical stimulation promotes angiogenesis and breast growth by promoting a pro-proliferative process. It was hypothesised that the use of the BRAVA® device would facilitate breast reconstruction by improving autologous fat graft retention rates. Without the use of the BRAVA® device, the current literature suggests a retention rate of around 60%-70% of the transferred fat graft at 3 months, with good long term maintenance of volume (Delay et al., 2009a). The suggested graft retention rate using the BRAVA® is approximately 82% (Khouri R.K., 2012). Initial clinical research indicated that women who complied with the procedure experienced an increase in breast volume, with 35–250 mL of lasting tissue growth (Khouri R.K., 2012). This volume decreased over time in the absence of fat grafting. The use of the BRAVA® device prior to autologous fat grafting has shown promise in terms of improving long term efficacy; however, only limited magnetic resonance imaging (MRI) studies performed by this group appear to support this hypothesis. In animal studies, increases in vascularity and cell proliferation have been demonstrated to be significant after mechanical deformation by a suction cup device. Khouri et al., reported in studies where the external expander was combined with autologous fat graft that graft retention rates reached 80% of the initially injected fat volume. He attributed the higher fat graft retention rate to the use of the BRAVA® external expander device to improve the vascularity of the host bed (Khouri R.K., 2012). Khouri et al. hypothesised that there is insufficient space in small breasts to allow for the dispersion of fat droplets from a fat graft (Khouri R.K., 2012). Khouri put forward the idea that the graft-to-recipient interface is

critical for revascularisation and that expansion of this interface allows better graft take. To date, there has been no prospective quantitative study assessing the efficacy of the BRAVA® device plus autologous fat grafting as a viable option for reconstruction after breast-conserving surgery and for total mastectomy defects. There are no randomised controlled trials assessing pre-expansion of the BRAVA® external expander device with subsequent autologous fat grafting versus fat grafting alone. It has not been shown objectively whether there will be an observable increase and maintenance of breast volume. Additionally, it has not been empirically demonstrated that patients treated with a BRAVA® device plus autologous fat grafting have comparable outcomes to patients treated with other types of surgical breast reconstruction. The outcomes which needed to be explored related to the level of patient compliance, whether outcomes related to patient factors such as previous breast radiotherapy and objective measurement of patient satisfaction with breast, psychosocial well-being, physical well-being in the chest area, and sexual well-being post-autologous fat grafting, objective measurement of improvements in breast volume symmetry.

Although an initial study by the surgeon who developed the BRAVA® system reported good long-term results, further studies with a larger number of patients are required to confirm the effectiveness of this system. In two letters to the editor, it has been previously noted that the device is uncomfortable to wear and it does not always maintain the seal that is necessary to produce the negative pressure required to expand the breast skin (Smith et al., 2002, Fuentes-Felix, 2003). Overall, its use by patients who have undergone breast-conserving surgery or total mastectomy has not been thoroughly investigated. This study aimed to assess whether the use of a BRAVA® device with autologous fat grafting is a viable

option for reconstruction of breast-conserving surgery and total mastectomy defects using the Miami protocol (Khouri R.K., 2012). Specifically, we sought to evaluate whether such patients are able to tolerate treatment with a BRAVA® device and whether there is an observable increase and maintenance of breast volume following the procedure

5.2 Methods

5.2.1 Ethics Approval Number

The Ethics Committee approved a study into the efficacy of the breast expanding device and autologous fat grafting (Southern Adelaide Clinical Research Ethics Committee approval number 321.13). All patients were given a participant information sheet (Appendix 5) and along with obtaining standard operative consent, consent to the purpose of research was also obtained (Appendix 6).

5.2.2 Funding

This research was supported by the South Australian Health and Medical Research Institute Beat Cancer Project Blue Sky Cancer Research Fund. The Grant application was entitled "*Pilot study of a breast expanding device and fat grafting for breast reconstruction after cancer*". The chief investigator for this study was Dr. Nicola Dean.

5.2.3 Recruitment

Since 2009, Flinders Medical Centre has offered autologous fat transfer for breast reconstruction and contour defect correction. This prospective pilot cohort study enrolled patients who had undergone partial or complete mastectomies in the Breast and Endocrine Surgical Unit at Flinders Medical Centre (FMC). Patients seeking breast reconstruction were offered traditional methods of breast reconstruction with expander / implants or autologous flap reconstruction, along with the option for BRAVA® expansion and autologous fat graft reconstruction. The following patients were excluded from the study: women who were shown to have an allergy to silicone (cup domes consist of silicone at skin level) during a 20-minute outpatient trial, women with untreated breast cancer, women considered at

high risk for breast cancer recurrence, and women who were pregnant or breast-feeding.

5.2.4 BRAVA® device

Tissue engineering and regenerative medicine have been born out of the need to enhance the body's ability to recreate and rebuild tissues and organs by combining cells, scaffolds, and biologically active molecules into functioning tissue with or without foreign biomaterials (Lancerotto et al.).

In tissue engineering, there are two general approaches (Cronin et al., 2004):

1) *Ex vivo* construct of scaffold which is then implanted *in vivo*;
2) Stimulation of the in vivo production of native tissues.

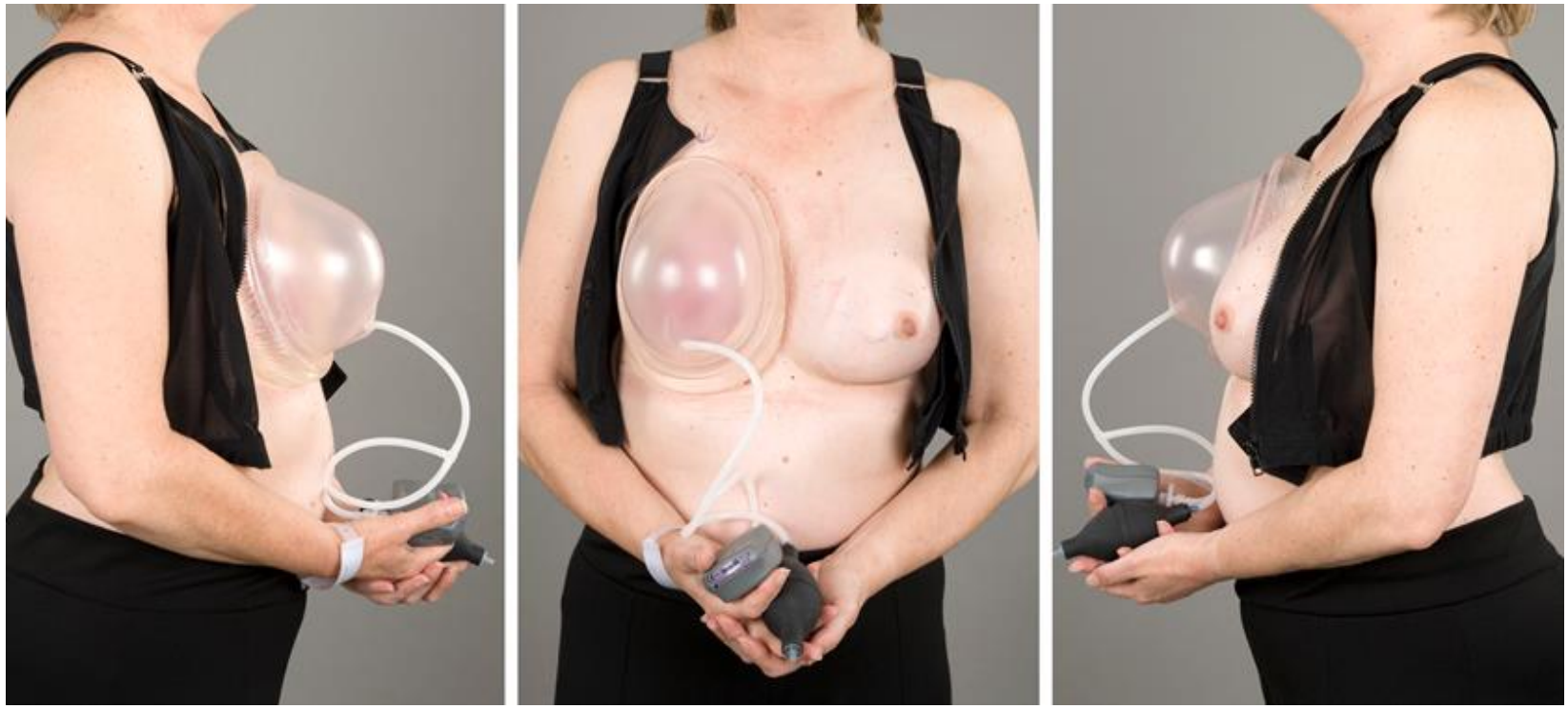
The BRAVA® device has been shown previously to trigger a proliferation of cells with mechanical stimulation (Heit et al., 2012). The process of skin deformation and strain and subsequent inflammation promotes signalling of cells to increase vascular permeability and 1-α growth factors, which results in cell proliferation of the epidermal and dermal layers, angiogenesis, and adipogenesis (Lancerotto et al., 2013). On two occasions, Dolderer has shown that new fat can form within a chamber around a vascular pedicle (Dolderer et al., 2007, Dolderer et al., 2011).

The BRAVA® was designed to be worn like a brassiere. Within its fabric, there are two 2 semi-rigid polyurethane domes that are placed around the interface with the skin through silicone gel-filled donut bladders. The bladders form an air-tight seal, helping to dissipate pressure and shear forces. A small, battery-operated, microchip-controlled mini-pump maintains 20 mm/Hg of negative pressure inside the dome. Preliminary tolerance of the device was first established in the outpatient

setting with a 20-minute trial. Generally, the use of the BRAVA® device should be painless, and if a patient experienced pain, they were advised to discontinue its use immediately. Ideally, the BRAVA® is worn for 10 hours per night for 28 nights prior to the autologous fat transfer procedure. Overall compliance was defined as at least 8 hours of wear per day for 4 weeks. Partial success constituted less than 80% compliance, but with enough skin expansion to facilitate some transfer of fat. Non-compliance leading to abandonment of the device was considered a failure.

Patients were also instructed to use the BRAVA® for 7 days post-operatively for 10 hours, provided they had no on-going pain issues. In theory, this post-operative period of wear promotes oxygenation to the transplanted fat (Uda et al., 2014). Appendix 4 shows the BRAVA® device diary patients were requested to maintain during its use. All women who underwent this procedure complied in maintaining entries in this diary. Pre-operatively, the BRAVA® device costs consumers $1995, not including the purchase of 4 sets of AA batteries. The cost is reduced to $1675 if 20 devices or more are purchased. The BRAVA® device can be used for repeat procedures. The Khouri Lipografter is single-use and costs $300. The reusable Khouri cannula was purchased for $180. A post-operative abdominal binder costs patients $170.

Figure 5-1 A patient wearing the BRAVA® device on the right side.



5.2.5 Autologous fat grafting

The same senior Plastic and Reconstructive Surgeon carried out all procedures. Patients underwent a single-stage moderate to large volume autologous fat graft as previously described by Roger Khouri (Khouri R.K., 2012, Harvey et al., 2005). The Khouri Lipografter is a device used in the Miami protocol and has FDA approval. It forms a closed loop, enabling large volumes of fat to be harvested, whilst maintaining sterility. The calibrated metal spring within the handle of the lipografting device allows the plunger of the syringe to be drawn outwards, generating low pressure suction of fat into the barrel of the syringe. It is connected to a silicone tube via a, non-return valve. The overall minimisation in suction force, as compared to a more conventional suction pump, theoretically minimizes cellular destruction and results in a high yield of whole cells. The KVAC-Syringe® is fully cocked by manually pushing down on its plunger. This unfurles metal springs which once unfurled add a constant withdrawing force on a 30ml plunger. After incisions are made in the abdomen, the Khouri cannula is inserted and fat harvesting begins. The Khouri Cannula is electro-polished and coated externally and internally with zirconium nitride. The manufacturer hypothesises that the coating of the cannula promotes reduced harm to adipocyte viability to promote smooth atraumatic fat harvest with reduced destruction of individual adipocytes.

The At-Valve (A-T Armaturen LTD.) is a simpler alternative to conventional 3-way stopcocks plus syringe or the use of multiple syringes. The use of the At-Valve's 1-way valves eliminates the need to constantly adjust the stopcock. Once fat is harvested into the 30 mL syringe, the AT-Valve prevents fat from being injected through the cannula, and instead it is directed down the silicone

tubing into a previously emptied 100 mL intravenous saline bag. The AT-Valve is rotated once the 100 mL saline bag is full, at which point the fat is injected from the 100 mL bag into the breast. This system minimises overall fat graft handling. Currently, a comparable fat grafting system, the LipiVage® system requires the direct injection of the filled 30 mL syringe into open-ended 10 mL syringes, a process that is time consuming and susceptible to spill hazards.

Khouri et. al. suggests in his clinical studies that the combination of the Lipografter and Khouri cannula in a close-looped system shortens operative times while improving patient outcomes (Khouri R.K., 2012). Therefore, we anticipated better fat graft retention rates over time.

Training in the use of the new equipment was undertaken with the aid of a representative of MediGroup Australia. As similar equipment has been used previously, all staff became proficient in the use of the new equipment during the period of its implementation on the first three patients.

As the fat graft was harvested, it was placed into 100 mL sterile bags and allowed to separate into layers.

Once it had been suspended for a minimum of 10 minutes, the aqueous component (local anaesthetic fluid, and blood) were decanted off, the fat graft removed from the bag into syringes, and the oil component left in the sterile bag. The syringes of fat were then injected into the recipient site (breast or mastectomy defect).

Given the contention in the literature regarding Adipose Derived Stem Cells (ADSCs) and potentiation of breast cancer cells as discussed in Chapter 2, the design for a “lipografter patient record” was initiated in consultation with a

specialist radiologist This was designed in conjunction with a professional medical graphic artist (Flinders Medical Centre Department of Medical Illustration and Media). The documentation of exactly where the autologous fat was injected and into which plane, e.g. subcutaneous or intramuscular (pectoralis major), would aid in identifying whether a radiological abnormality was related to an area of injected autologous fat graft. Appendix 1 shows the first version of the lipografting record that was designed to be included in the patients' case notes. Appendix 2 and 3 are follow-up versions and were designed once it became clear in clinical practice that the Appendix 1 version was found not to provide enough space to list all recorded injections on each breast, so this was changed to a single breast per A4 page

With all systems of fat grafting there is the potential for post-operative "lumps" or radiological abnormalities. Although areas of fat necrosis and oil cysts are benign, they may still trigger investigation with mammogram, ultrasound, fine needle aspiration for malignant cells, or a punch biopsy for histology of any lesions that are considered suspicious. This process of investigation is an important consideration, as it may cause stress and anxiety for the patient and has resource implications. The instigation of a lipografting record to accurately document site of fat grafting was to assist radiologists and breast clinicians in these circumstances.

Intraoperatively, an arterial blood pressure transducer was set up by the attending anaesthetist and placed into the breast at the beginning and the end of the operation to assess the interstitial pressure of the breast.

At the conclusion of the procedure and while the patient was still asleep, a compression garment was placed on them to reduce oedema and the risk of post-operative haematoma.

Figure 5-2 Lipografter, AT valve (2 x size), and harvest cannula.

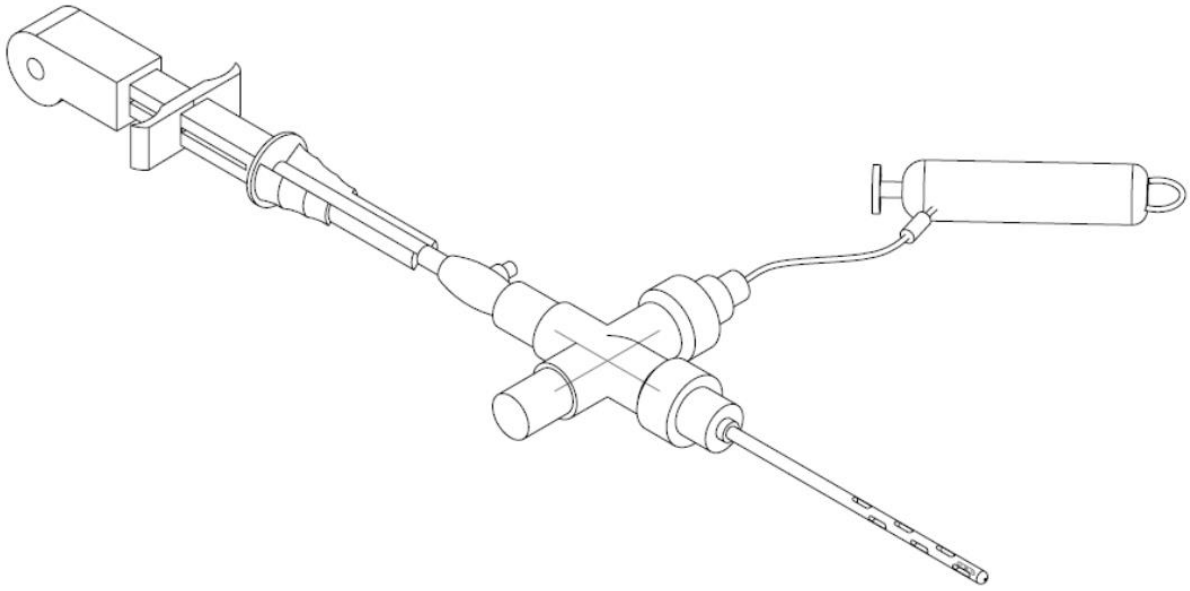
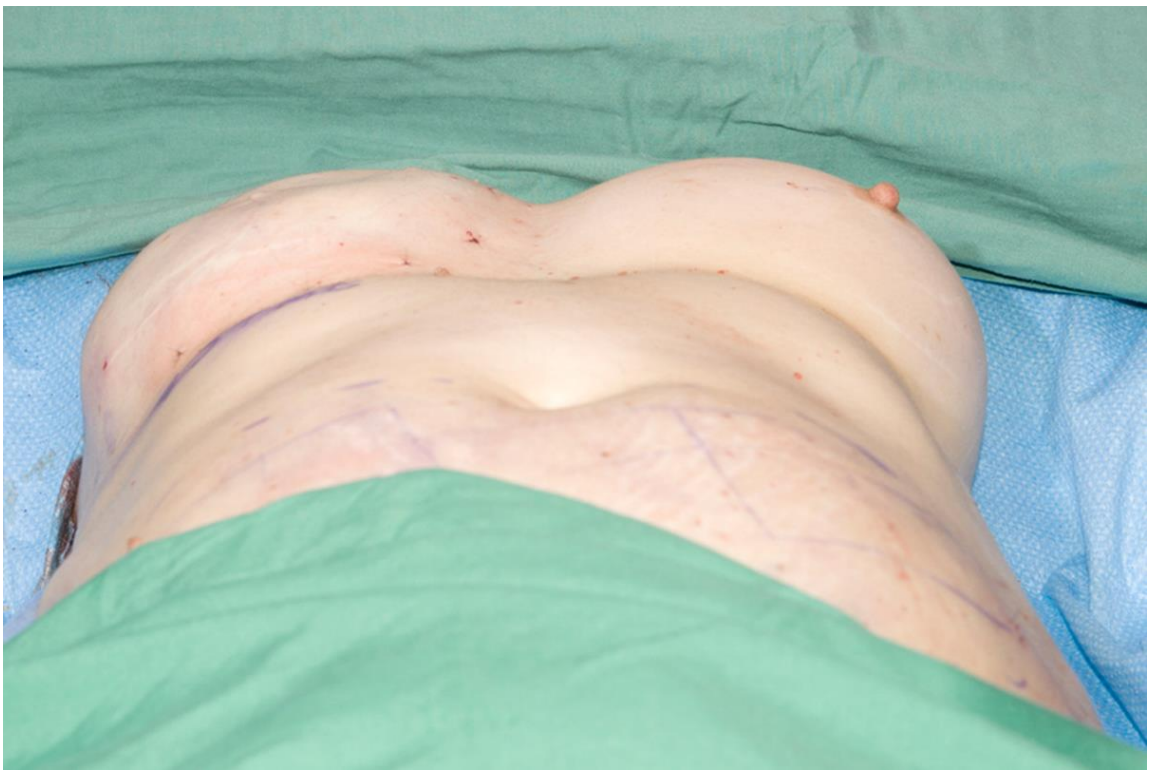


Figure 5-3 Suspending the fat graft to separate the fat from other injection fluids.

Figure 5-4 Final case results of a woman who underwent fat grafting to the right breast. The left breast was not treated in this case.



5.2.6 The BREAST-Q™

The BREAST-Q™ is a validated, patient-reported outcome measure developed by the Memorial Sloan Kettering Cancer Institute and the University of British Columbia (Pusic, 2003a). The BREAST-Q™ was developed in 2009 to elicit and quantify patient perception of outcomes following augmentation, reduction, and reconstruction (Pusic, 2009). It contains 36 items with raw scores being converted to summary scores out of 100 for three satisfaction and three well-being domains using "Q-score" software (Pusic, 2003b). The key domains for this study were 'Satisfaction with Breast', 'Physical Well-being Chest', and 'Psychosocial Well-being', and 'Sexual Well-being' (Pusic, 2009). Since the advent of this tool in 2009, our practice has implemented its use. It has also served as a governance tool to audit and improve services. As the BREAST-Q® was developed with patient-focusgroups using the Rasch methodology; the questions resonate strongly with patients, resulting in high compliance in terms of completion and a good dataset. The raw questionnaire data were converted using the Q-Score program found at the developer's website (www.BREAST-Q.org). The program provided summary scores for each BREAST-Q™ scale, ranging from 0-100, and gathered relevant information regarding patient satisfaction and quality of life.

The results of the BREAST-Q™ for the study cohort were compared to the results of other breast reconstruction patients at the Flinders Medical Centre and to related published data. A 'successful outcome' was judged as one that was within 10 percentage points of the mean score for Flinders Breast Reconstructive Service patients in the domain of 'Satisfaction with outcome'

5.2.7 3D Laser Scanner and Breast MRI

Objective outcomes of treatment were assessed using a 3D laser scanner in a proven methodology for measuring breast volume, previously used for FMC studies (Approval number 269.08).

3D laser scans were performed prior to treatment, the day prior to fat grafting, and then 3 and 6 months following the autologous fat transfer using the protocol previously described in the validation study by Yip J. et al. The volumes of the operated breast and untreated normal breast were measured. The normal side was used as the control. In total, 22 breasts were volumetrically analysed using the 3D laser scanner.

The specific outcomes measured with the 3D scan technique were:

- Comparison of injected volume of fat to the increase in scanned volume (pre- to post-op). The percentage of fat retention was determined by the volume injected divided by the volume measured during follow-up.
- Comparison of the fat volume increase at 3 months post-operatively compared to 6 months post-operatively.
- Breast volume symmetry (volume of smaller breast / volume of larger breast) with a volume ratio of 0.8 or greater was defined as acceptable symmetry.
- Pearson correlations between the following were investigated:
    - Body mass index (BMI) and fat graft retention rates.

- Intra-compartmental pressures in the breast (measured during the operation by an arterial blood pressure transducer) and fat graft retention rates.

Breast MRI was undertaken at the same time as the 3D laser scanning, as breast MRI is the current gold standard for volumetric assessment. These were "non-contrast" MRIs (without the injection of gadolinium) and scans were taken pre-fat grafting and 6 months post operatively. The same senior radiologist interpreted all of the MRI images to eliminate inter-rater variability. For the same reason, the scanning and analysis of the 3D images was conducted by a single clinician who was blinded to the MRI volume result at the time of analysis..

A cost comparison was also undertaken to compare this new technique with the traditional methods of breast reconstruction.

5.2.8 Comparison of Autologous Fat Grafting with other reconstructive procedures (expander/implant reconstruction and autologous flap reconstruction)

In the Flinders Medical Centre theatre database, a search was conducted to explore the number of reconstructive procedures that were conducted over a three year period (2013-2015) along with their respective operative times. This particular time period mirrored the time period for recruitment and follow-up of the enrolled participant group. The purpose of this search was to compare operative times between autologous fat grafting and the more traditional methods of reconstruction.

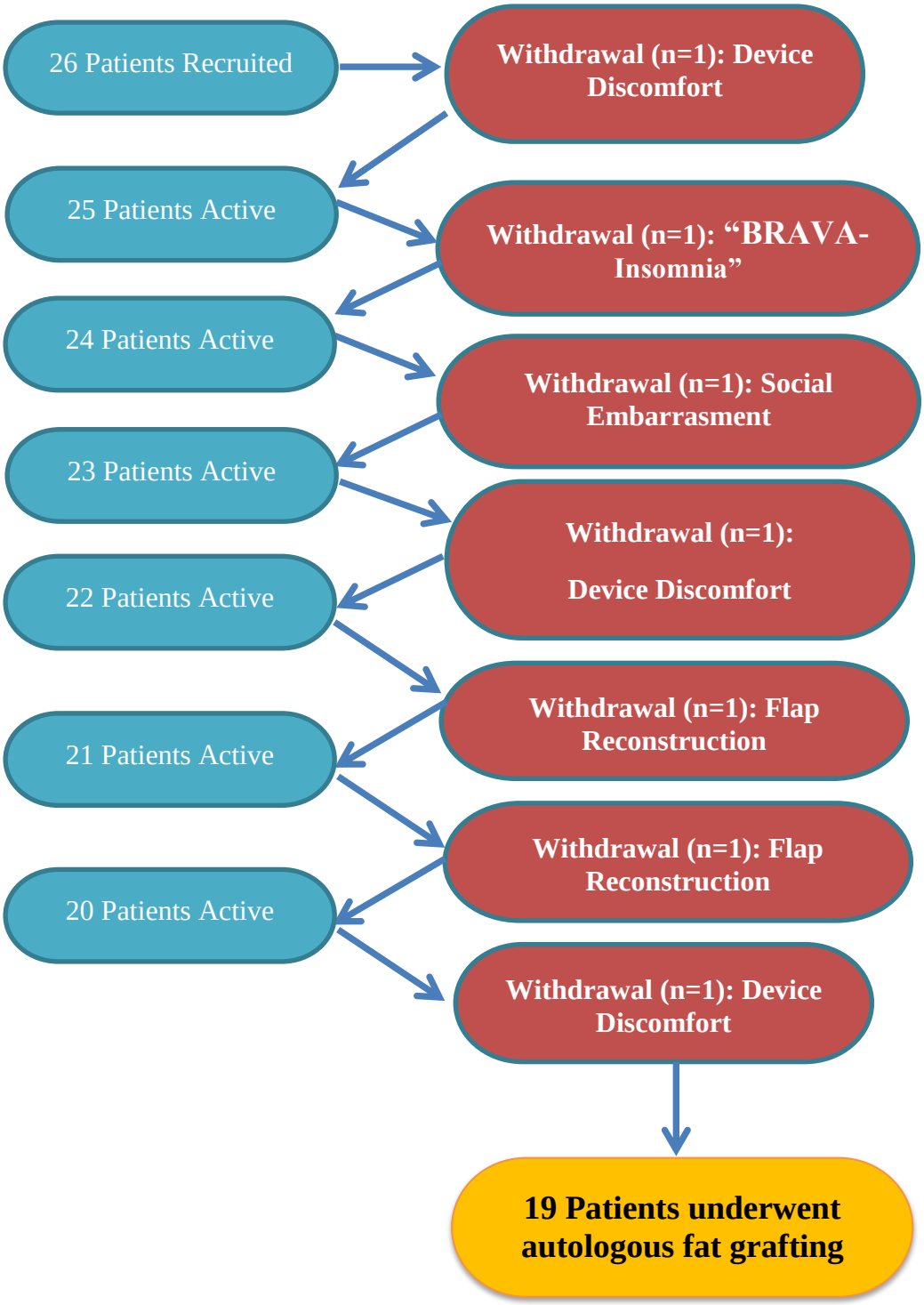
In order to calculate an estimate of the cost gain per hour for each breast reconstruction, data pertaining to medications and discharge scripts were collected from the inpatient records. Patients were divided into respective groups and the number of medications used was ascertained from the inpatient medical charts. The cost of each medication was then determined via the hospital pharmacy and the number of uses of each medication was multiplied by its cost. The same process was carried out for discharge scripts. The average amount of money spent on inpatient and discharge medications was recorded for each breast reconstruction group.

5.3 Results

The number of patients recruited for this study was 26, and there were 7 withdrawals (28%). Reasons for withdrawal included the following: 1 because of social embarrassment; 1 patient who decided against reconstruction; 3 had device discomfort; 1 patient who opted for a more traditional method of reconstruction; and 1 patient who experienced “BRAVA-insomnia” for 8 nights due to difficulties maintaining a seal. The patient recruitment is demonstrated in figure 5.

A total of 19 patients remained in the study. The median age was 53.8 (41-67 years). Four cases were bilateral and 15 were unilateral (22 breasts in total).

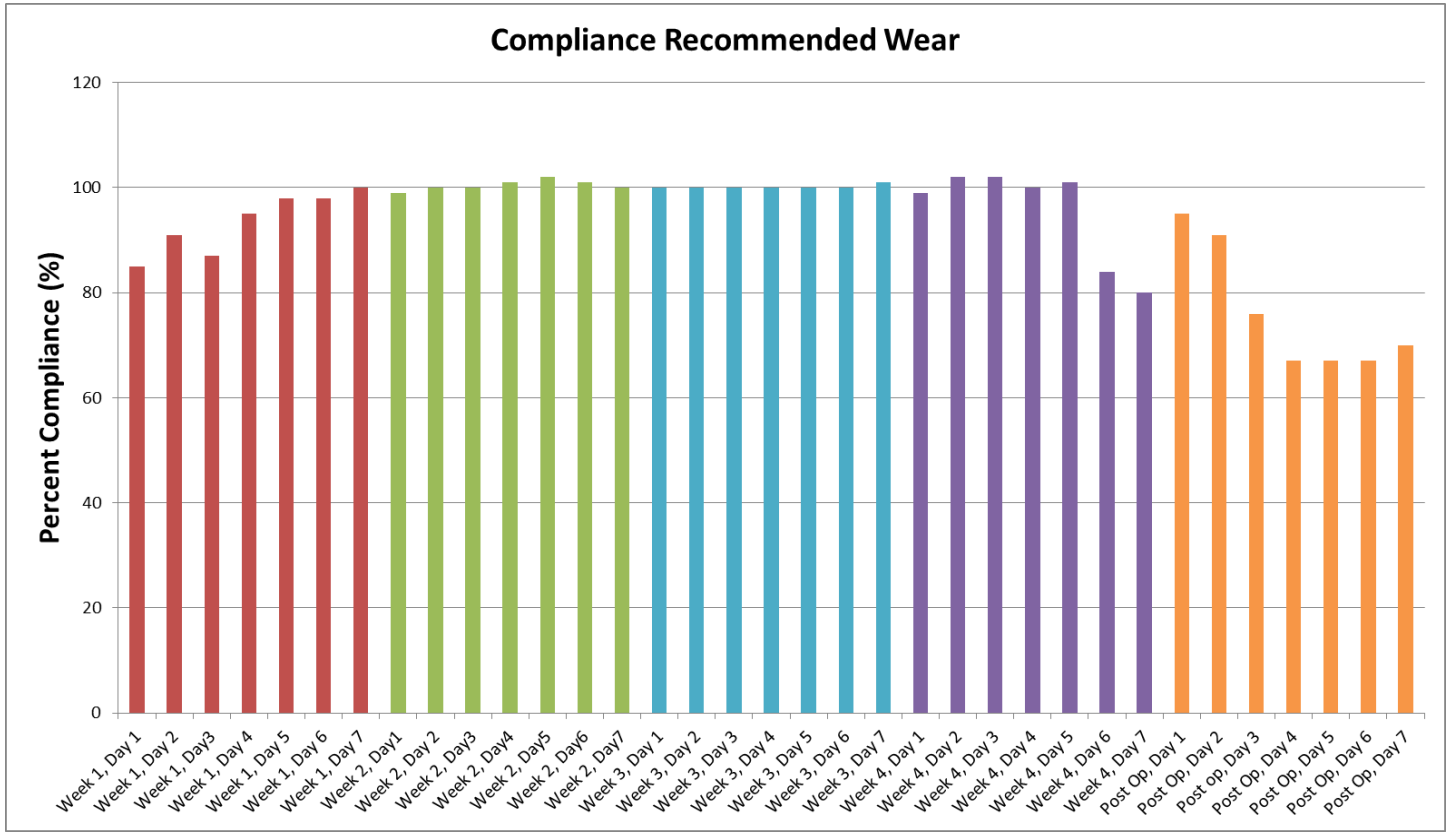
Figure 5-5Flow diagram: patient recruitment versus withdrawal



5.3.1 Patient compliance

The overall compliance with the BRAVA® device is summarised in Figure 5-6. All patients in the pre-operative wear group were considered compliant with a greater than 80% rate of wear. However, the average wear in the post-operative period constituted non-compliance. Reasons for non-compliance in the post-operative period included difficulties maintaining a seal in the silicone footprint owing to the change in contour and general discomfort. In 2 cases, the formation of blisters occurred; one patient experienced this prior to her fat grafting treatment but overcame it with dressings. The other patient had not experienced blisters prior to the operation.

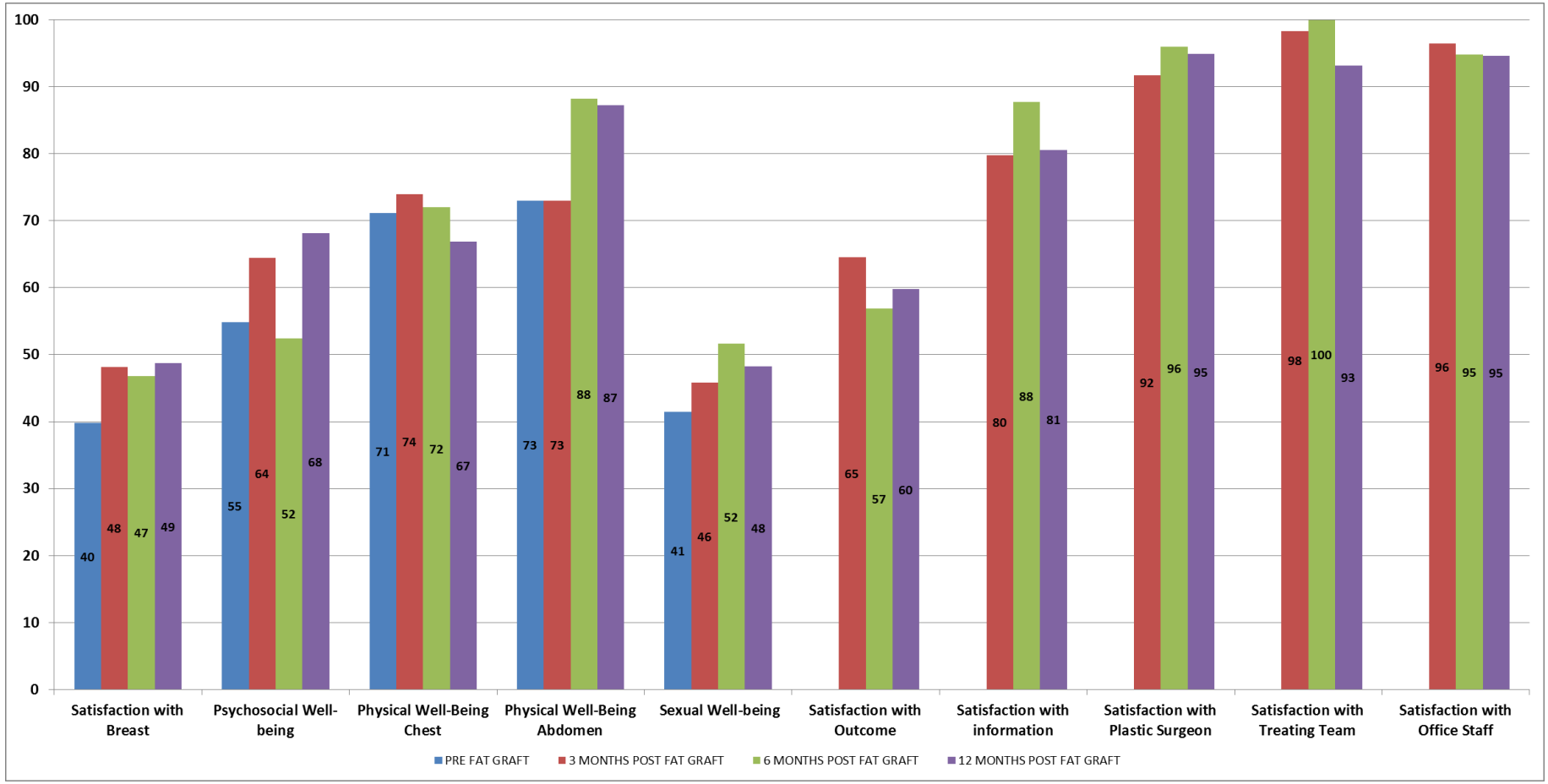
Figure 5-6Compliance of BRAVA device wear versus days (pre operative and post operative periods)



5.3.2 BRAVA® expansion

During the course of the study, it was noted that patients were experiencing volume increase benefits from the BRAVA® device. For those patients who consented, an additional 3D laser scan was performed prior to wearing the BRAVA® and compared with the volume observed after 28 days of BRAVA® use and prior to autologous fat grafting. It was feasible to do this in a total of 6 patients. . The average increase in the 12 breasts that were measured was 67.8 mLs (-17 to 110 mLs). The average volume symmetry ratio increase was 0.15.

Figure 5-7 BREAST-Q^{TM} Questionnaire- quality of life scores versus operative periods (centred numbers are average scores)

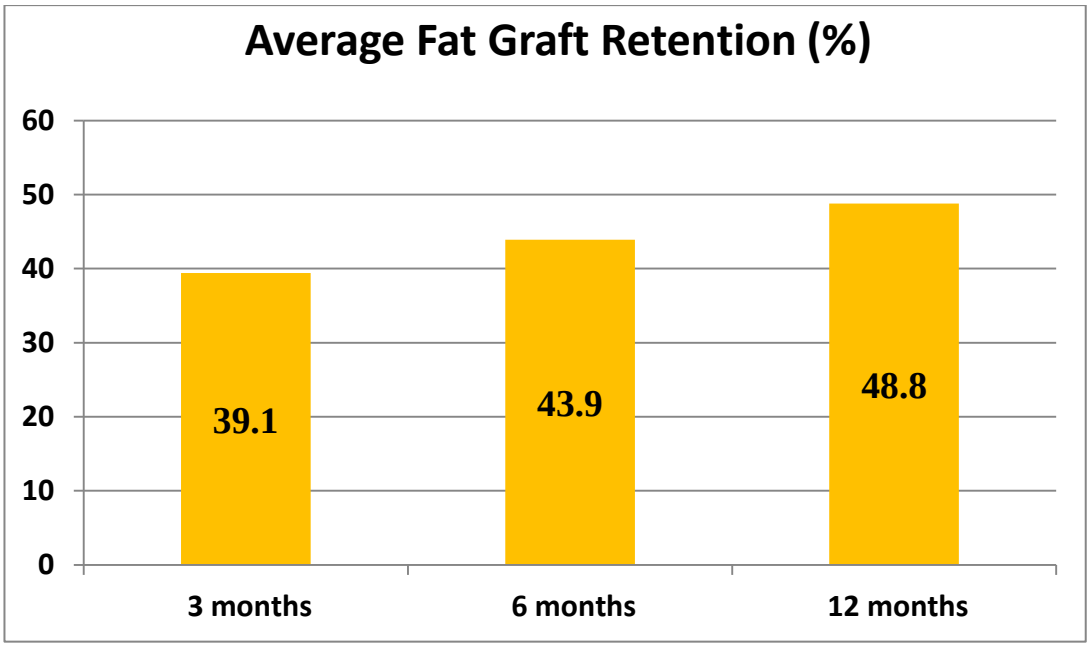


5.3.3 Autologous Fat Grafting and Graft retention rates

The average amount of fat engrafted 270.4 mLs (98- 490 mL). The amount of fat that was retained is represented in figure 8.

MRI and 3D laser scans were comparable and this is discussed further in Chapter 6. At the 12-month post-operative assessment, 2 patients had withdrawn from the study and 1 patient wished to undergo expander/implant reconstruction, leaving 16 patients to undergo bilateral 3D scan evaluation. The average maintenance of breast volumes at 12 months was 48%.

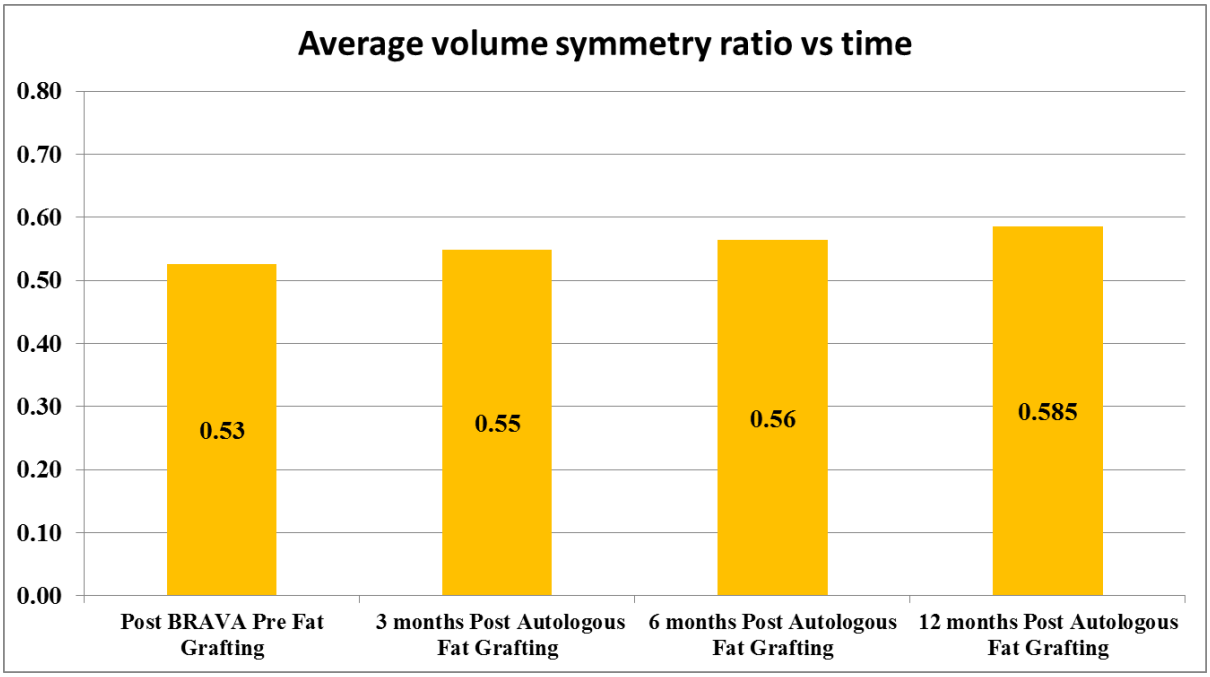
Figure 5-8 Average fat graft vs period post autologous fat grafting.



5.3.4 Volume Symmetry Ratio

Comparing the 3D scan volume symmetry ratios over time, a ratio of 0.53 was seen pre-operatively, with ratios of 0.55 and 0.56 at 3 and 6 months after fat grafting, respectively. Breast MRI volume symmetry ratios were 0.48 pre-fat grafting and up to 0.51 at 6 months post-fat grafting. None of the patients achieved acceptable symmetry, previously defined as a symmetry ratio greater than 0.8. Only 2 patients achieved a symmetry ratio improvement of 0.2. Improved symmetry between 3 and 12 months may be explained by a single patient, LF; the case involved the removal of a left-sided breast implant and mastopexy, which was aimed at improving her breast symmetry.

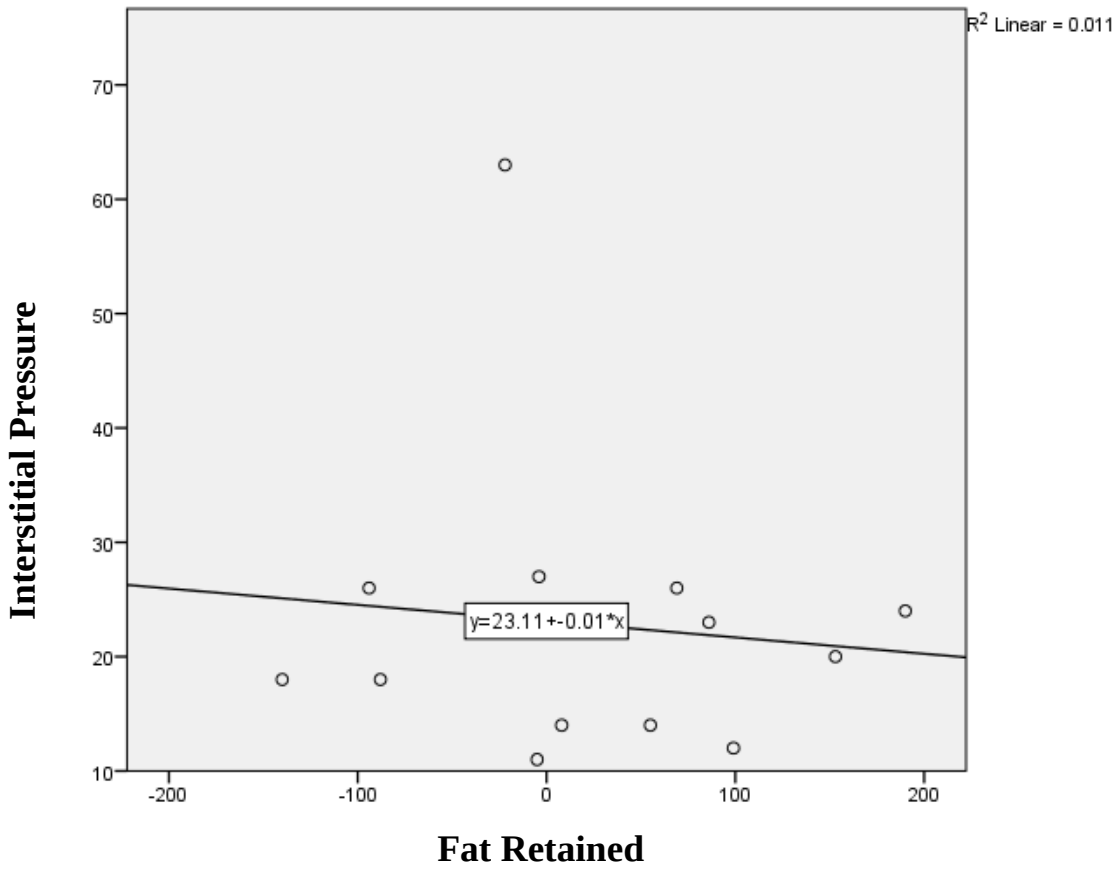
Figure 5-9 Average volume symmetry ratio versus fat grafting postoperative 3D volume scans



5.3.5 Interstitial pressure and fat graft retention rates

The measurements of intra-compartmental pressures at the end of the procedure were recorded and showed an average pressure of 22.7 mmHg (11- 63 mmHg). The Pearson correlation below demonstrates a decreasing linear relationship between compartment pressure and the amount of fat retained, although this was not significant.

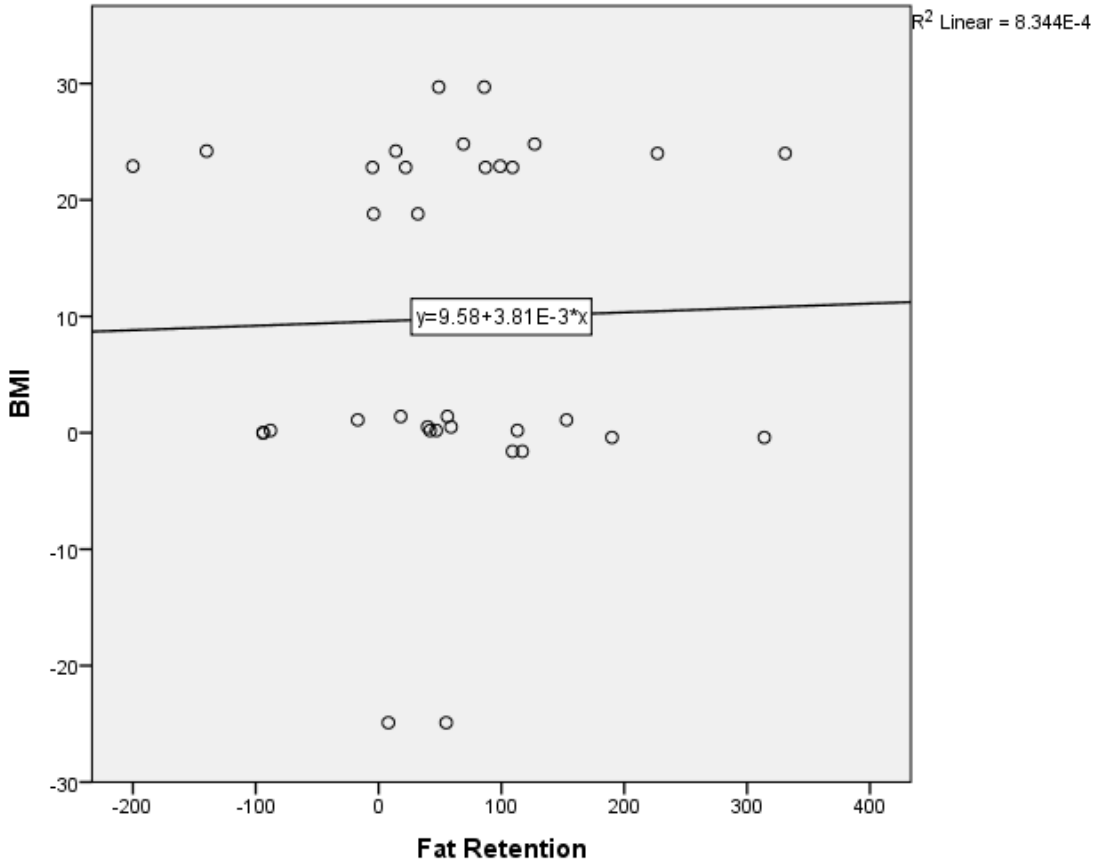
Figure 5-10 Interstitial pressure vs fat retained



5.3.6 BMI and fat graft retention rates

The average BMI was 25.2 at 3 months and 25.9 at 6 months post-fat grafting. At 12 months, there was an average increase of BMI by 1.1 points to 26.3 in the whole group. The Pearson correlation between BMI and graft retention rates showed a linear increase in retention rates with increasing BMI, although this was not significant.

Figure 5-11 Body mass index (BMI) versus fat retention



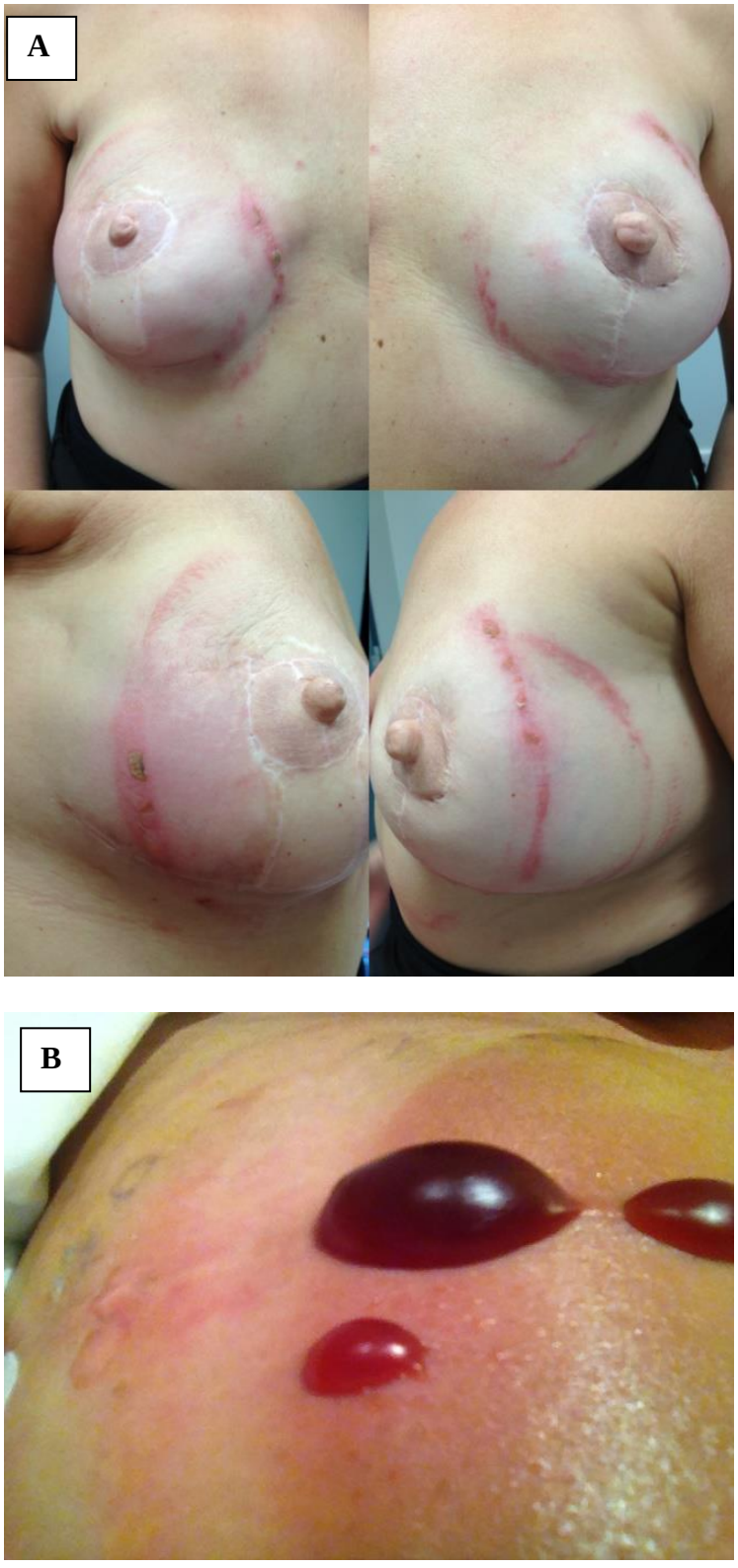
5.3.7 Radiotherapy and fat graft retention rates

Fifteen patients had undergone radiotherapy prior to the procedure, while 4 patients did not. At 12 months, the average retention rate was 51% for those who had undergone radiotherapy, yet it was 37% for those who had not. Unfortunately, 2 patients withdrew prior to the 12-month follow-up, as they had both had undergone radiotherapy and experienced minimal clinical benefit from the autologous fat grafting. Had they been included at the 12-month mark, the result may have been less than 51%. At the 6-month follow-up, 1 patient had a mere 4% rate of graft retention in the right breast, with 27% retention in the left breast. Given this low retention rate, she withdrew from the study prior to the 12-month follow-up.

5.3.8 Complications

Skin irritation was the most common complication for 11 patients out of the 18 (61%). These patients experienced some form of irritation ranging from erythema and icterus to bullous lesions that contained either serous fluid or were haemorrhagic. One patient agreed to a biopsy as the cause of the skin blisters was unknown. A 4 mm punch biopsy revealed epidermis that was separated from the underlying dermis along the dermo-epidermal junction, with focal necrosis and overlying compact orthokeratin with focal parakeratosis. Within the superficial and mid-dermis, interstitial and perivascular inflammatory cell infiltrate comprising lymphocytes admixed with scattered neutrophils and eosinophils was observed. Minor pigment incontinence was also noted. This histological diagnosis seemed to be in keeping with contact dermatitis.

Figure 5-12 Contact Dermatitis (A) and Haemorrhagic blisters (B) from BRAVA® device wear.



In another study by Ho Quoc and Delay (Ho Quoc and Delay, 2013), a similar experience with blister formation was reported with near identical photos taken during their trial.

It was necessary to convert incisions in the breast from 14-G needle incisions to 11 blade stab incisions to ease the passage of the injection cannula. An 11-G bone marrow biopsy needle was sourced for the remaining incisions.

Mechanical device failure occurred in three cases; either the lipografter spring or the cannula attachment broke, requiring a new device to be used intraoperatively. The company replaced these devices at no cost. During the fat graft harvest, blockages within the lipografter made it difficult at times for the coils to retract and provide adequate suction. Fat or lubricating gel was spread onto the metal spring to ease the action of the spring-driven plunger.

Once the fat graft had filled the barrel of the Khouri lipografter the plunger was pushed to transmit the fat graft into the 100 mL sterile bags via the AT valve and tubing. It was noted that significant force on the plunger was required in order to transmit the graft along the length of the tubing to the bag. The tubing was then cut in half to decrease its length in order to decrease any resistance to the flow of fat per Ohm's law (Ohm, 1827).

The quality of the lipoaspirate tended to diminish as the procedure continued, relating to either an increasing amount of blood in the aspirate or diminished efficacy of the lipografter.

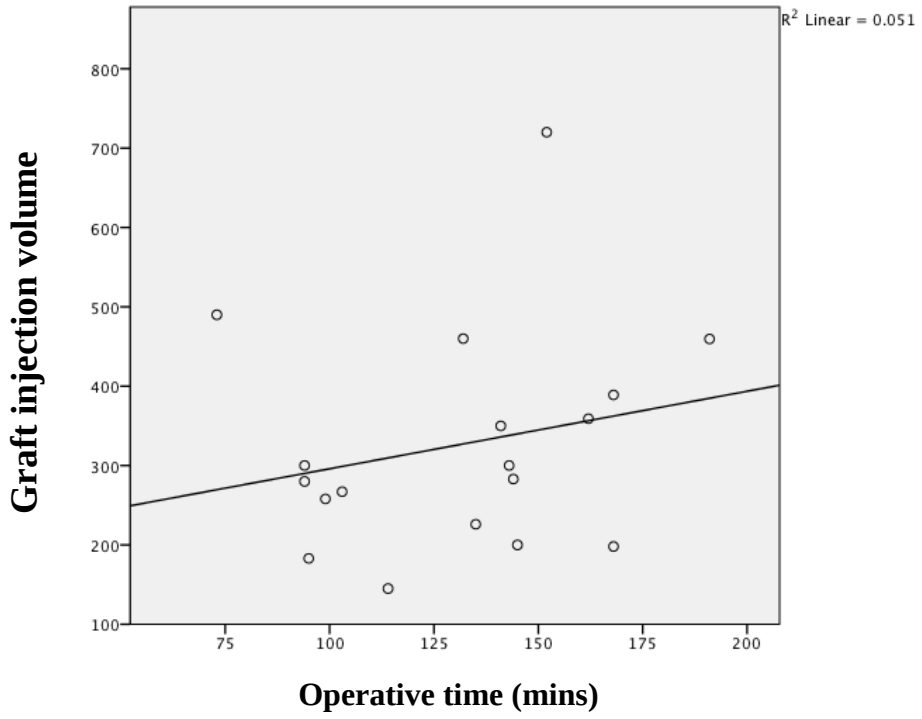
There was minimal donor site morbidity from lipoaspiration. Although the literature reports that repeated harvest in one site can result in pitting

and dimpling of the skin this did not occur in our series of patients. Two different patients had abdominal haematomas that were managed conservatively and did not result in long-term sequelae. In terms of recipient site complications, two women underwent ultrasound for masses post-fat grafting; one ultrasound demonstrated benign inflammation, while the other demonstrated a benign-type lesion. This lesion was biopsied and was determined to be fat necrosis. There was no recurrence of breast cancer in any participant at 12 months follow up.

5.3.9 Comparison of autologous fat grafting and traditional breast reconstructive procedures

The average operative time was 129.4 minutes (73-191 minutes) for autologous fat grafting. Interestingly, there was no correlation between the graft injection volume and the operative time of the harvest. However, there was a slightly increased linear relationship between volume harvest and time. Notably, on average, the unilateral fat grafting procedures took longer than the bilateral procedures. However, on average, larger volumes of fat graft were harvested during the unilateral procedures (276.8 mL) compared to those during the bilateral (239.6 mL). Unsurprisingly, when comparing average harvest times for unilateral fat grafting with the expander insertion, the time taken for fat grafting was longer, as the volume of the expander is fixed and there is no fat graft harvest time. However, the average bilateral fat grafting although the number of patients spanning this time frame is small.

Figure 5-13 Graft injection volume versus operative time.



Eighteen patients were discharged the same day with one patient staying overnight as per anaesthetics request for hypotension secondary to dehydration. For those patients who had retrievable data on the cost of inpatient and discharge medications.

Operation	Inpatient Meds (n)	Price (Aus)	Discharge (n)	Price (Aus)	Total (Aus)
Autologous fat grafting	19	$1.73	19	$5.92	$7.65
Expander/implants	43	$38.5	57	$14.81	$53.31
Latissimus Dorsi	13	$104.4	8	$16.73	$121.13
TRAM	10	$94.1	12	$4.23	$98.33

5.3.10 Repeat Procedures

Eight women agreed to additional fat grafting procedures. Although this was not initially included in the plan of the study, the ethics proposal was revised after 3 months when it became apparent that minimal effects were obtained from a single fat grafting procedure for some patients. Clinical equipoise dictates that the necessary judgment be exercised when the desired outcome is not being achieved. At follow-up appointments, women were offered further fat grafting treatments once they had completed the 12-month follow-up period of the study. Those women who wished to have different procedures, e.g. expander/implant or latissimus dorsi reconstruction, were not included in the 12-month data collection analysis, as they were not re-scanned once they underwent their supplemental breast reconstruction procedure.

5.4 Case of BRAVA® wear and autologous fat grafting for total mastectomy breast reconstruction

Mrs. LF had previously undergone total mastectomy via a rotation flap approach having previously undergone submammary augmentation with silicone implants. She wished to undergo BRAVA® and autologous fat grafting preferentially over traditional methods of breast reconstruction. She was deemed to be at low risk of breast cancer recurrence by her breast cancer surgeon. She underwent breast implant removal and breast reduction between 3 and 6 months, and had improved scores in all domains.

Figure 5-14 Case of total mastectomy BRAVA® and AFG breast reconstruction: BREAST-Q™ scores versus time

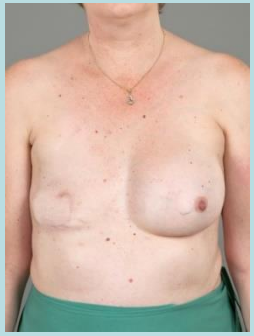
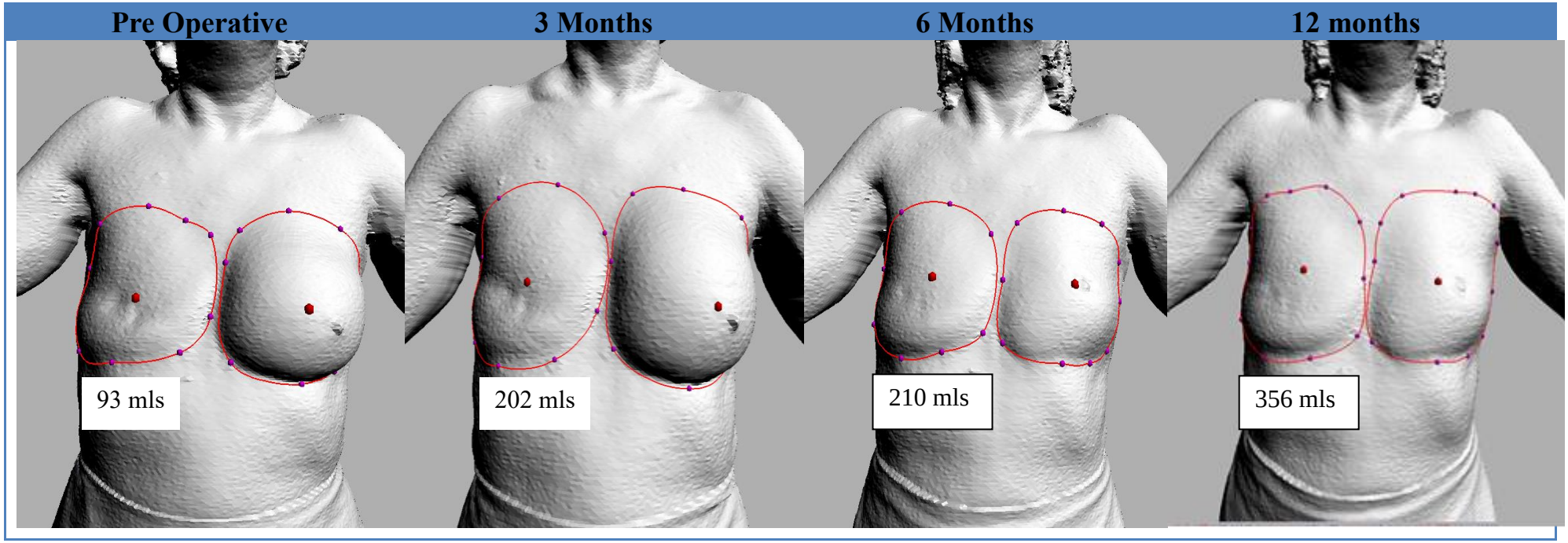
2D photography & BREAST-Q Domains over time	Pre operative	3 months	6 months	12 months
				
Satisfaction with Breast	28	52	53	58
Psychosocial Well-being	42	42	32	58
Physical Well-being Chest	53	53	60	55
Physical Well-being Abdomen	46	90	100	92
Sexual Well-being	34	40	45	47

Figure 5-15 Case of total mastectomy BRAVA® and AFG breast reconstruction: fat graft volumes versus time as measured by the 3D laser scanner

Mrs. LF's fat graft volumes increased over time, particularly between months 6 and 12, which may be partly attributable to weight gain during this time period.



5.5 Case of BRAVA® and autologous fat grafting for breast conserving surgery breast reconstruction

Mrs. RL had previously undergone breast-conserving surgery. The lateral margins of the excision of her breast cancer were wide (hence the reason she presented with a defect), and while her tumour was a reasonably large mass, it had been excised several years prior and she had completed adjuvant radiotherapy. Her breast oncological surgeon deemed her low risk for recurrence. Given that there are few options for small defects of the breast such as this, she wished to undergo a trial of BRAVA® expansion and autologous fat grafting. Although her scores improved from pre-operative to post-operative in all domains, she did not wish to undergo another BRAVA® and autologous fat grafting procedure, which may have further corrected the breast-conserving surgery breast defect. This may be attributed to her improved psychosocial well-being in the 12-month period since her BRAVA® and fat grafting treatment.

Figure 5-16 Case of breast-conserving surgery BRAVA® and AFG breast reconstruction: BREAST-Q™ scores versus time

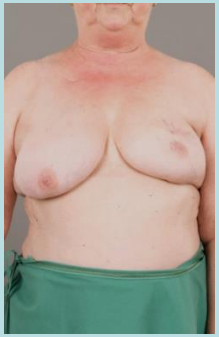
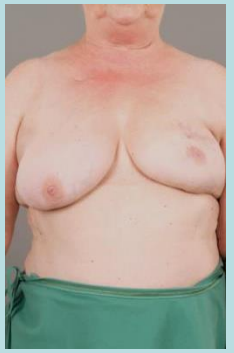
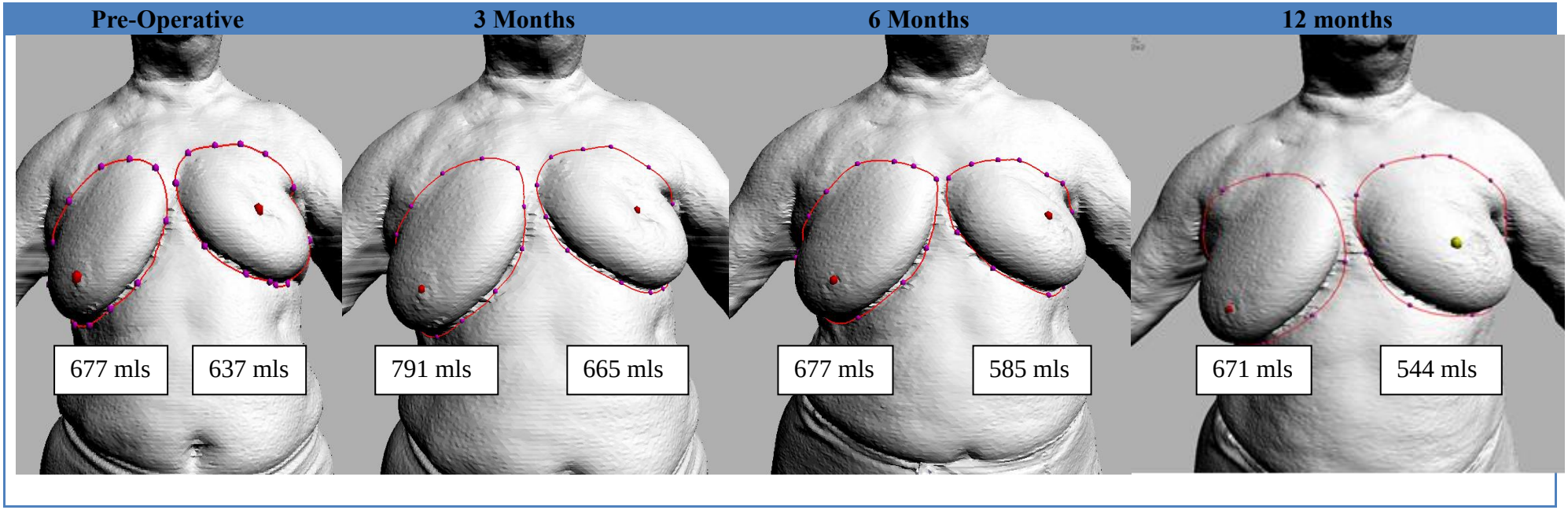
	Pre-operative	3 months	6 months	12 months
2D photography & BREAST-Q Domains over time				
Satisfaction with Breast	**22**	**16**	**39**	**44**
Psychosocial Well-being	**14**	**23**	**47**	**65**
Physical Well-being Chest	**57**	**54**	**63**	**68**
Physical Well-being Abdomen	**35**	**29**	**100**	**100**
Sexual Well-being	**0**	**16**	**34**	**40**

Figure 5-17 Case of breast -conserving surgery using BRAVA® and AFG breast reconstruction: fat graft volumes versus time as measured by the 3D laser scanner

Mrs. RL initially experienced an increased volume of fat graft on the left side (treated breast) compared with the pre-operative volumes. However, as time progressed, she had decreased scores in both her treated and untreated breasts. The most likely explanation for this may be either weight loss, or for the treated breast, the loss of fat graft owing to the effects of radiotherapy on tissues as discussed previously in Chapter 2.



5.6 Discussion

Breast expansion and the BRAVA® device

Traditional breast expansion methods involve the use of internal silicone expander., These are empty silicone rubber shells with a filling port and a footprint similar to that of the removed breast, that are inserted into the breast area after a woman has undergone mastectomy for breast cancer. Once inserted underneath the pectoralis major muscle, the silicone expanders can be injected with saline in the outpatient setting to increase the size of the neo-breast by stretching the overlying tissues. Once the expander has stretched the skin (over a 4-6 week period) and created a pocket, it is then replaced with a silicone implant. Expanders develop a pocket in which to position an implant where there would otherwise be no room. This type of breast reconstruction procedure can be performed immediately following mastectomy or in a delayed manner. Complications that are associated with silicone implant based reconstruction include implant extrusion, palpability, visibility, and capsular contracture. The longer term complications of silicone implants such as capsular contracture and rupture have taken time to become evident to the public, but now that many women understand that silicone implants may cause complications and need to be removed years after they are inserted, there have been some who are anxious about the potential risks involved and who are looking for alternatives for reconstruction. One controversy in particular which raised the public awareness of long term implant complications concerned the implants produced by Poly Implant Prosthese (PIP) that were used in 400,000 women from 2001 (Health, 2013). In March 2010, marketing and use of PIP

silicone implants was suspended because of the increased rupture and bleeding rates reported with PIP silicone implants as compared to other types of implants. .

In order to offer an alternative to women and to overcome some of these concerns, Roger Khouri invented the lipografter equipment and the BRAVA® device (Khouri R.K., 2012). The concept was aimed at avoiding the need for internal expanders and silicone implants. Autologous fat grafting is much less invasive than traditional breast reconstruction procedures, with much smaller incisions and reduced overall donor/recipient site morbidity. Initially designed for cosmetic patients, Khouri extended the BRAVA® device's use to include women who had undergone both skin sparing and nipple sparing mastectomy. Kosowiski et al.'s "Clinics in Plastic Surgery" article defines 3 groups of women who have undergone mastectomy that are considered suitable candidates for recruitment to undergo tissue-engineered autologous breast regeneration with BRAVA®-assisted fat grafting (Kosowski et al., 2015). These are women who are undergoing either immediate breast reconstruction or delayed breast reconstruction, as well as women who have previously undergone breast-conserving surgery and radiotherapy. Immediate reconstruction requires mastectomy of the cancer-affected breast, followed by the harvesting of the fat graft from the abdomen or thighs, and then injection of the adipose tissue into the breast area to replace the section of the breast that contained the tumour and was removed. This could be considered unsafe, as clear oncological clearance via histopathological analysis has not yet been undertaken. Pathological analysis occurs once the breast specimen, which contains the cancer, has been excised and is sent to a surgical pathologist for analysis. Furthermore, the patient has not yet undergone completion of treatment in terms of

radiotherapy and endocrine therapies. To this end, there is no assurance that the adipose-derived stem cells introduced into the breast will not interact with cancer cells that have not yet been cleared from the native breast tissue. For the women in our study, a period of at least 6 months following completion of the breast cancer clearance process had passed prior to enrolment, and they were all considered at low risk for recurrence by their breast cancer surgeons.

To date, the literature on the BRAVA® device includes case reports and small series. The majority of these studies were retrospective in nature and although they provide important information on the use of autologous fat grafting and the BRAVA® device, not all of them have quantitative data using validated outcome measures (Smith et al., 2002, Fuentes-Felix, 2003, Schlenz and Kaider, 2007, Zocchi and Zuliani, 2008, Del Vecchio and Bucky, 2011, Khouri R.K., 2012, Ho Quoc and Delay, 2013, Uda et al., 2014, Hammer-Hansen et al., 2015, Uda et al., 2015, Kosowski et al., 2015). The current study aimed to use validated quantitative measures such as the MRI scanning, 3D laser scanning, and the BREAST-Q™ questionnaire (Pusic, 2009). Mestak describes a case in which a woman underwent bilateral breast reconstruction using autologous fat grafting and the BRAVA® device and used the device for 2 months prior to each episode of fat grafting and for 1 month post-operatively (Mestak and Zimovjanova, 2012). Their patient required 3 fat grafting sessions. A French article by Ho Quoc and Delay was based on a prospective trial of 21 patients' post-total mastectomy (with or without radiation) that sought to observe compliance rates with the device (Ho Quoc and Delay, 2013). They observed a high overall compliance rate and no patients abandoned the device. Complications from the device included minor occasional skin irritation with erythema and pruritus. With conservative treatment, patients continued the use

of the BRAVA® device. Unfortunately, this article did not discuss their outcomes related to the fat grafting procedure. Schlenz and Kaider trialled the BRAVA® device without fat grafting on 40 women who desired breast augmentation for cosmesis (Schlenz and Kaider, 2007). They were followed p for 10 months after discontinuing the BRAVA® device (Schlenz and Kaider, 2007). Patients wore the device for 11 hours per day for 18.5 weeks (mean) with a median volume increase of 155 cc at 304 days follow-up, suggesting that there is maintenance of volume over time using the device. In women with intact native breast tissue who have not undergone mastectomy, the device was concluded to be effective; however, breast volume was measured with the Grossman-Roudner device, which is less accurate than either a mammogram or Archimedes' principle, and has not been compared to laser scanning (Kayar et al., 2011). Khouri et al.'s study was a prospective multicentre trial including 81 patients who underwent BRAVA® and fat grafting that showed very promising results (Khouri R.K., 2012). However, the author initially stated in the methods section that the patient would undergo a volume assessment using MRI at 6 months post-op, but not all patients were scanned at this time in actuality. The volume increase as measured in the 12 breasts in the present study showed minimal increases in volume pre- and post-BRAVA® use. Outside of the expansion benefits, other potential factors such as the creation of a 'fibro vascular scaffold' that promotes better vascularisation of grafted fat were not investigated in this thesis. In a mouse model, Heit et al. applied a silicone dome to the back of mice at a suction pressure of negative 25 mmHg and demonstrated that this yielded a 2-fold increase in the subcutaneous layer consistent with MRI findings, an increased proliferative rate in cells, a net 2.2-fold increase in the number of adipocytes, and an increase in vessel diameters with a 1.9-fold increase

in vessel density (Heit et al., 2012). This finding appears to be consistent with an inflammatory process that, along with oedema, might promote angiogenesis, which in turn might promote adipogenesis when coupled with lymphatic stasis. This process was shown by Harvey et al. in his in vivo study (Harvey et al., 2005).

Regarding the practicality of the BRAVA® device, the protocol for its use has changed over time; at first, the maintenance of a constant pressure of -60 mmHg was advised, and then the sport box became used to create cyclical pressure. Likewise, the BRAVA® went from being worn pre-operatively for 2-4 weeks with 24-48 hours of uninterrupted wear, to being worn for more prolonged periods of time (Monticciolo et al., 1994). This variability may be attributable to the individual patient's tolerance levels, with 2 weeks of wear for 24 uninterrupted hours representing the minimum wear time described in the literature. This approach was not suitable in the context of this research study as all of the patients were expected to undergo the same operative conditions. Zocchi and Zuliani described "Bicompartment Breast Lipostructuring"(Zocchi and Zuliani, 2008) and emphatically applauded the use of the BRAVA® device and autologous fat grafting in their set of cosmetic patients. This was also found to be the case with Del Vecchio and Bucky who used the BRAVA® device in 25 cosmetic reconstruction cases totalling 46 breasts. They concluded that a mega-volume fat graft (>300 cc) injection occurring in less than a 2-hour operation, confers long lasting results. Likely, the native breast tissue expands more easily and provides a well-vascularised environment in which to inject the autologous fat graft, thereby increasing fat graft retention rates and decreasing the likelihood of fat necrosis (Del Vecchio and Bucky, 2011).

The combination of the BRAVA® external expansion and fat grafting for breast reconstruction after breast cancer surgery is an appealing concept owing to its minimally invasive nature, shorter operative times, autologous nature, and overall cost effectiveness. Women who chose this procedure had not found autologous flap reconstruction or implant reconstruction to be appealing choices because they did not wish for extensive surgery with a long recovery period, or they were uncomfortable with the idea of having a foreign body implanted. The selection of this particular cohort of patients did not affect compliance with the BRAVA® device or the follow-up requirements in the context of this study, including more post-operative visits, the maintenance of a diary, and the completion of questionnaires at intervals. High compliance was previously noted by Uda et al., who suggested that after the importance of the use of the device was explained, women were willing to comply with the instructions for wearing the device (Uda et al., 2014). Similarly, the patients in our study were told of the potential benefits of wearing the device, which was that it would assists autologous fat graft retention by providing a well-vascularised bed for the fat graft.

Previously, Khouri's patients were considered ready for fat grafting once they had achieved an increase in volume of greater than 2.5 times the pre-expansion volume from the BRAVA® device (Monticciolo et al., 1994). However, it is unclear how exactly this was measured which makes it difficult to tailor the mount of fat required from harvest in order to correct defects and achieve symmetry. Another difficulty associated with the BRAVA® fat grafting protocol pertained to unclear nomenclature, as centrifugation is usually determined by revolutions per minute for a fixed time period rather than '15g'(Monticciolo et al., 1994). In order to overcome this lack of specificity, all 100 mL sterile bags were suspended in the

same way for a period of approximately 15 minutes or until the fat graft had clearly separated into layers that enabled the surgeon to decant off the blood and aspirated fluid (Roger Khouri conference presentation, Plastic Surgery Congress, Gold Coast, Australia 2013).

5.6.1 Fat grafting

Fat grafting is not a novel procedure and has been used increasingly in the last decade for contour defect correction and the enhancement of breast volume following breast cancer surgery (Bonomi et al., 2013, Delaporte et al., 2009, Petit JY, 2011, Khouri R.K., 2012). Breast reconstruction using autologous fat grafting for women who have undergone total mastectomy defects is feasible though the long-term efficacy and best methods for reconstruction have yet to be established (Groen et al., 2016). There is also a consensus in the literature that fat grafting to the breast in breast-conserving surgery and total mastectomy patients is a viable procedure (Spear SL, 2005, Kronowitz et al., 2006, Delay G., 2009, Delay et al., 2009a, Kling et al., 2013, Bland and Altman, 1986). However, in the case of total mastectomy reconstruction, more than one session of fat grafting may be required (Monticciolo et al., 1994), and there remains an issue regarding the efficacy of the procedure relating to fat graft reabsorption. To better understand reabsorption after grafting, Peer's study injected autologous fat into the rectus sheath of humans and then, after a few months, excised parts of the graft at intervals to be examined microscopically (Peer, 2007). Some cells survived, while others were replaced by histiocytes and fibrous tissue. Overall, 50% of the fat graft weight remained at one year. Small grafts may be easily reabsorbed and larger grafts can exhibit a high rate of liquefaction, necrosis, and cyst formation. Delay documented a 30-40% reabsorption rate of fat at 3 months post-grafting, with good long-term outcomes for

up to 10 years (Delay G., 2009). Peer reported scientific evidence supporting successful long-term maintenance of the volume of autologous fat after grafting (Peer, 2007). Two other studies reported improvements in breast size, and Delay reported that 75% of their patients were happy with their results (Delay G., 2009, Khouri R.K., 2012).

Missana et al. sought to determine fat graft viability following reconstruction with implants, and as a cosmetic adjunct after LD and TRAM flap reconstruction with and without radiotherapy in 74 cases of women who had undergone breast-conserving surgery (Missana MC, 2007). MRIs were taken pre-operatively to establish a reference 'adipose map'. MRIs were then taken 3 months post-operatively to evaluate graft evolution and cytosteatonecrosis.

There have been several technological advances regarding autologous fat grafting in order to optimise the harvest and processing of the graft prior to injection into the breast (Perez-Cano et al., 2012, Ferguson et al., 2008). Prior to the advent of the Khouri lipografter, other utilized instruments included the LipiVage system (Ferguson et al., 2008). This system employs a mechanical suction of approximately 508 mmHg, whereas the Khouri lipografter has a suction of 250 -350 mmHg (Harvey et al., 2005). The question of whether or not this alters the yield of viable adipocytes in the graft remains to be explored.

The operative time for fat grafting was comparable to the insertion of expanders, and the volume of fat graft injected was comparable to the starting volumes of the internal expansion volumes. However, more fluid can be injected into the expander in an outpatient setting, whereas approximately 60% of the fat graft can be expected to be reabsorbed. Expanders are therefore considered more

reliable when the intention is to establish a desirable breast volume.

The operative time for bilateral breast reconstruction using autologous fat graft was not that much more than the injection time for a single breast. This contrast the time it takes for more traditional methods of bilateral reconstruction, eg. LD or TRAM reconstruction. Once the planned volume of fat graft has been harvested from the abdomen or thighs, both breasts can be injected with the autologous graft simultaneously by two separate surgeons. The corollary is that traditional methods usually reach the desired breast volume in a single procedure, whereas autologous fat grafting often requires repeat procedures. In one case by Hammer-Hansen, the total volume of injected fat was 957 cc, but the injection of this volume spanned 7 autologous fat grafting sessions (Hammer-Hansen et al., 2015).

When patients were consulted in the Flinders Medical Centre Breast Reconstruction Clinic and offered more traditional methods for breast reconstruction (mainly transverse rectus abdominis musculocutaneous and latissimus dorsi reconstructions), some found a 5-7 day hospital stay followed by a 6-8 week recovery period to be daunting. What appeals to women about the BRAVA® and autologous fat graft reconstruction is the minimally invasive nature of the procedure with small incisions and the fact it can be carried out as a day procedure. Smaller incisions in autologous fat grafting result in decreased donor and recipient site morbidity, which reduces the overall risk of complications. The simplicity of the procedure is *quid pro quo* for the need for repeat procedures to achieve the desired breast reconstruction volume and this should be explained to women during the informed consent process.

5.6.2 Interstitial pressure

Further injection of a fat graft once interstitial pressures have reached 20 mmHg is not recommended. In our experience, the interstitial pressure routinely reaches higher values after only small injections of fat graft. The concern that has been raised regarding the restriction of capillary perfusion by interstitial pressures seems justified. However, practically speaking, fat can only be injected until the pressure forces the graft back through the incision site. The average intra-compartmental pressure was 22.7 mmHg, which was slightly higher than the 20 mmHg previously recommended by Khouri. Whether or not this relates to the decreased fat graft retention rates needs to be investigated in larger studies. Generally, while injecting fat graft into the breast, there was a fine line between increasing the intra-compartmental pressure and achieving the complete injection of the required volume of fat graft. When the intra-compartment pressures within the breast or in one particular tunnel of injection were high, the fat graft leaked back through the injection holes.

One technique for the even distribution of fat is to inject with ‘microribbons as to not form lakes’, but that is also very difficult to manage given that this technique is blind. However, attempts were made to avoid injecting aliquots in the same place by distributing the fat graft in a fanning pattern. Further investigation as to how the injection pattern affects graft retention is necessary.

5.6.3 Fat graft retention and symmetry

The slight increase in fat graft retention rates in patients who had undergone total mastectomy within our study performed less well compared with patients who had undergone breast-conserving surgery. Despite the over-correction of the fat grafting and pre-operative compliance with the BRAVA® device, graft retention rates were lower than anticipated. However, they were comparable to the 39% retention rate reported by Choi et al., as well as the 40% rate reported by Spear et al. (Choi et al., 2013, Spear SL, 2005). The use of the BRAVA® device in the post-operative period was suggested by Uda et al. to improve the partial pressure of oxygen thereby improving oxygenation of the fat graft and recipient bed (Uda et al., 2014). The increase in the partial pressure of oxygen was demonstrated in a graph, but unfortunately the method at which the author arrived at the measurements was not documented (Uda et al., 2015). If partial pressure of oxygen from the BRAVA® device improves graft retention rates, this may also explain the poor retention rates given the lower compliance in our cohort. Conversely, since BRAVA® expansion may function by producing oedema and inflammation, this may not assist grafted fat due to the creation of an ischaemic environment during the post-operative period. However, given the lower compliance in our group of women, this is less likely to be the sole cause for the reduced fat graft retention rates.

The set of patients in our study demonstrated poor compliance with the device during the initial week. The main complaint was insomnia ('BRAVA-insomnia' was the phrase coined by one patient), which may explain some of the reduced graft retention rates. Still, regardless of overall pre-operative compliance levels, graft retention rates should still have been higher than demonstrated overall.

The slight BMI increase in some patients over the study period may explain increased fat graft retention and volume symmetry rates, as the fat is susceptible to the same humoral effects as native abdominal fat, which may also explain improved volume symmetry ratios. Although, 1 particular case may have skewed the average symmetry score after she had removal of a prosthesis and mastopexy. Volume symmetry was not improved to greater than 0.8 in many cases, and since only 2 patients experienced a 0.2 point increase, it is further argument for the need for multiple autologous fat grafting sessions in order to achieve optimal breast symmetry. Whether the BRAVA® device improves breast volume symmetry is a question that requires further exploration.

5.6.4 Complications

Various methods of fat grafting exist, including the LipiVage® system (Genesis Biosystems, Texas, USA) which was previously being used at our institute. In our experience, there have been problems with the quality of the cannulae and the effectiveness of the system in light of failures of the filtration system. In one instance, damage to the filter allowed a fat graft to be lost down into the Clement Suction Unit. Although this particular issue was not experienced with the Khouri Lipografter and cannulae, there were occasional problems with the connection between the harvesting cannula and the A-T Valve, leading to failure and a need for replacement.

The majority of women who wore the device described skin irritation ranging from erythema, pruritus, dermal bullae, and general discomfort, which often resulted in insomnia. In more severe cases, patients had bullous lesions that were of great concern for both the patients and clinicians. This higher than expected skin complication rate while wearing BRAVA® has previously been reported by Uda et

al. (Uda et al., 2014). Uda's team prescribed hydrocortisone cream, whereas the patients in our study used dressings (Tegaderm and Duoderm combinations), which helped form a protective barrier between the silicone adhesive layer and skin. Other methods for prevention included the use of Dermatix® (Hanson Medical Inc., Western Australia, Australia) by one patient. Skin issues relating to the use of the device have been under-reported in the literature as explained by Uda et al. (Uda et al., 2014). Previous use of this device resulted in 20 ulcerated infections in one study, and this finding correlates well with our experience. The Hammer-Hansen study also observed a case of skin irritation that resulted in blistering which caused pain, insomnia, and increased visits to wound clinics (Hammer-Hansen et al., 2015). That patient was initially prescribed sedatives, antihistamines, topical steroid cream, and a Comfeel dressing, and later had to reduce the duration of BRAVA® wearing to 2 hours per day. Ultimately, the patient's skin irritation was healed only by the cessation of use of the device, though a skin prick test for a silicone allergy was negative. Fortunately, with appropriate dressing regimens and early intervention, there were no major complications such as skin cellulitis requiring intravenous antibiotics or sepsis. Given the obliging nature of the cohort of women who had undergone breast cancer treatment, they were very compliant with dressings and ointment regimens, and the majority were able to persevere until it was time for the autologous fat grafting. The advice given to women regarding a skin care regimen is provided in Appendix 5.

In terms of using the BRAVA® device for expansion, it has been stated that it can reconstruct the breast without additional scars or incisions (Khouri R.K., 2012). In our observation, in the absence of continued BRAVA® wear after the first post-operative week, the volume that was able to endure long-term post-

reconstruction was from the autologous fat graft. The degree to which the BRAVA device enhances the autologous fat grafting procedure remain to be thoroughly investigated. In order to inject the fat into the breast, incisions in the abdomen and thighs are necessary depending on the harvest site. Incisions must also be made in the breast area in order to inject the fat into the pectoralis major and subcutaneous breast pocket areas.

Although the advent of this device is a step in the right direction toward new tissue regeneration techniques, we observed the failure of the device's ability to create a fibrovascular scaffold that would lead to satisfactory and long lasting fat graft retention. Although a more positive effect may be clinically observable in women who have remaining native breast tissue (cosmetic cases) or in women who have had a level 1 sector resection for grade 1 or 2 breast cancer, it is difficult to observe any breast expansion in women who have had undergone total mastectomy. This difficulty becomes even more pronounced in women who have also undergone radiotherapy. In some cases, the long horizontal scar created by the total mastectomy necessitated a Z-plasty procedure prior to BRAVA® and autologous fat grafting. This was performed in an attempt to recruit additional skin to the area and seemed to assist the expansion process. For all women attempts were made at using the Percutaneous Aponeurotomy and Lipofilling (PALF) or Rigottomi procedure to try to break up scar tissue within the breast envelope which was present from either the mastectomy procedure or radiotherapy, or both (Rigotti G., 2005). Post-operatively, the patients who had undergone radiotherapy and experienced chest wall tightness noted an improved range of motion and softening of the skin. In fact, two of the patients who were previously considered unsuitable for expander/implant-based reconstruction became able to undertake the procedure.

This is likely more attributable to the regenerative effect of the adipose-derived stem cells than to the BRAVA® device, and in those patients, 'mega volumes' of reconstruction were not achieved and so whole breast reconstruction with fat grafting alone failed. BRAVA® pre-expansion is a novel approach to enhancing the outcome of autologous fat grafting, which has been proclaimed as a useful alternative to breast-conserving surgery and total mastectomy autologous breast reconstruction. Unfortunately, although it has been touted as a minimally invasive and patient-friendly option for women who wish to regenerate their lost breasts (Monticciolo et al., 1994), this was not found to be the case in the present cohort study. To date there are no trials comparing BRAVA® and AFG with AFG alone and it is still not known whether BRAVA® contributes to the autologous fat grafting procedure. An ideal randomised control trial may include the following groups:

1) Mastectomy and delayed breast reconstruction with BRAVA® expansion only. The contralateral native breast without reconstruction as a control;
2) Mastectomy and autologous fat grafting only. The contralateral native breast without reconstruction as a control;
3) Autologous fat grafting and BRAVA® expansion. The contralateral native breast without reconstruction as a control.
4) Autologous fat grafting with full fraction (mature and pre-adipocytes). The contralateral native breast without reconstruction as a control.
5) Autologous fat grafting with full fraction (pre-adipocytes only). The contralateral native breast without reconstruction as a control

In the patients who had undergone radiotherapy, there was a noticeable clinical improvement in the suppleness of the previously radiotherapy damaged skin. This was noted by both clinicians and patients, and has been reported as a common finding in the literature as discussed in Chapter 2. Indeed, fat grafting and its application for tissue rejuvenation following radiotherapy damage and burns have been previously described by Klinger et al. (Klinger et al., 2008). Fat grafting introduces adipose-derived stem cells into an area that has previously undergone surgery with concomitant inflammation and fibrosis coupled by radiotherapy damage. Adipose-derived stem cells may assist tissue regeneration by improving the pliability of the dermis and epidermis. This could be beneficial in cases where radiotherapy damaged tissues restrict the stretching of the skin and the insertion of expanders is not an option owing to increased risk of implant extrusion. This benefit was demonstrated by one case in the present study involving a woman whose skin had been previously unsuitable for an expander that became pliable after fat grafting. She was then deemed suitable for expander/implant breast reconstruction. It is expected that fat graft retention will be reduced in a hypoxic environment, as mature adipocytes initially survive by diffusion of the surrounding tissue once injected. However, according to Suga et al., it may be the pre-adipocytes, or as named in their study, adipose-derived stem/progenitor cells that actually survive (Suga et al., 2010). In a severely hypoxic environment, the presence of dead cells increased, including adipocytes, but elevated expression of hypoxia-inducible factor 1α and fibroblast growth factor 2 was also found (Suga et al., 2010). This is discussed further in Chapter 7.

The presence of detectable fat necrosis in only two cases in the present study was a less common finding than expected given the number of breasts that were

injected with autologous fat grafting, and whether this can be attributed to the BRAVA® device is unknown. In Chapter 2, the rate of fat necrosis is described as 4.7%, which is the average of all cases currently in the literature. This rate was less than expected. In the current study, a comparable fat necrosis rate of 4.3% was found. A study comparing fat necrosis rates between autologous fat grafting only and women who undergo BRAVA® plus fat grafting would need to be conducted in order to compare findings.

While there were no recurrences of cancer during the study period, these patients will continue to be followed up on an annual basis indefinitely. None of the existing literature discusses the relationship between the volume of fat grafting and oncogenesis, and it focuses instead on the proximity of the fat graft to the excised tumour. This may have more impact in terms of potentiating cancer recurrence. For this reason, a fat grafting record was kept for each patient that included where exactly the fat was injected (subcutaneous or pectoralis major) and the volumes injected. In the event of a recurrence in the future, the volume, proximity, and previous breast cancer excision characteristics will be able to be reviewed in detail.

5.6.5 Patient-reported outcome measures

The results of our study suggest that women who have undergone BRAVA® external expansion prior to autologous fat grafting have marginally improved outcomes in the domains of 'Satisfaction with Breast', 'Psychosocial Well-being', 'Physical Well-being Abdomen', and 'Sexual Well-being' compared to their pre-treatment status. BREAST-Q^{TM} scores are summarised in Figure 2. There was improvement in the domains of 'Satisfaction with Breast', 'Psychosocial Well-being', 'Physical Well-being Abdomen', and 'Sexual Well-being' when comparing pre- versus post-operative questionnaires. When the pre-BRAVA® and

autologous fat grafting scores are compared to those at 12 months after the procedure, all domains demonstrated improvement with the exception of decreased scores between 6 to 12 months in the domains of 'Satisfaction with Outcome' and 'Physical Well-being Chest'. Between 6 and 12 months, 2 patients had fat necrosis cysts investigated with ultrasound and this appeared to decrease their scores in this area.

Scores for satisfaction with information, the plastic surgeon, treating team, and office staff all remained over 80 and were stable throughout the 12-month period.

Although the scores for women who have undergone BRAVA® and fat grafting were improved compared to pre-operative values, the post-operative scores are lower than for women who have undergone autologous flap breast reconstruction. In the study conducted by Dean and Crittenden (Dean and Crittenden, 2016) which reviewed the BREAST-Q scores of 343 women who had attended the Flinders Medical Centre breast reconstruction service, which spans a 5 year period, the flap reconstruction group demonstrates higher scores overall. For BRAVA and autologous fat grafting the post-operative score for satisfaction with breast was 40 compared to 64.92 in Dean's study (Dean and Crittenden, 2016). The same was true for psychosocial wellbeing (55 vs 71.47), physical wellbeing chest (71 vs 74.78) and sexual wellbeing (41 vs 54.17).

5.6.6 Cost analysis

Fat grafting for breast reconstruction took approximately 2 hours, as opposed to 6 hours for the traditional methods. This shorter operative time and reduced inpatient stay greatly reduced hospital costs. In the current climate, costs

associated with breast reconstruction require consideration. A microvascular autologous flap reconstructive procedure can take 6-12 hours, leaving hospitals to wonder whether they are fiscally advantageous in the long-term. R Khouri attempted a cost analysis of the autologous fat grafting and BRAVA® external expansion.

In the United States, Alderman et al. noted in their cost analysis that reimbursement for surgical time was highest for delayed tissue expander placement ($1977.7/hr) and lowest for immediate TRAM flaps ($327/hr), with LD flaps drawing a negative margin (-$398). Although TRAM flaps may offer good results to patients, the lack of sound financial return for these procedures makes expander implant procedures more appealing. The same can be said for autologous fat grafting, which has comparable operative times with associated decreased professional revenue and a reduction in the overall costs that need to be allocated to breast reconstruction. Rough estimates as to the breast reconstruction procedure cost gains per hour demonstrated similar findings. The cost benefit to the hospital in our patient cohort was positive for expander/implant reconstruction, with latissimus dorsi reconstruction resulting in the least amount of financial gain. With the addition of costs for staff and perioperative care, it is likely that these operations may run into the negative each fiscal year. Findings were similar for the use of inpatient and discharge medications. The cost of medications for latissimus dorsi reconstruction patients was the highest, while the cost for expander/implant reconstruction patients was the lowest. Implementation of an operation such as autologous fat grafting, which is likely to be financially comparable to expander/implant reconstruction in terms of operative time, is likely to benefit patients, health care system, and tax payers.

5.6.7 Limitations

The limitations of this study include the small sample size and heterogeneous group of women. We sought to investigate the use of this device in the context of a pilot study, so we investigated it in a preliminary context. The heterogeneity in the group enabled us to observe the use of the BRAVA® device and fat grafting in a wide spectrum of patients who might opt for this combination for breast reconstruction. Another limitation is the admixture of patients in our cohort; although small correlations could be made with the amount of graft retention in radiotherapy versus non-radiotherapy patients, these findings need to be explored further in larger studies

Conclusion

The current study suggests that women who undergo BRAVA® and autologous fat grafting do show slight improvements compared to baseline in terms of quality of life. In the use of the BRAVA® external expansion as an adjunct to autologous fat grafting, the factors that contribute to fat graft retention need to be explored further. High graft loss and the need for multiple procedures, particularly in women who have undergone total mastectomy have been reaffirmed in this study. Fat grafting continues to prove its value in reversing fibrosis from radiotherapy damage, but the oncological safety of autologous fat grafting in these patients needs to be determined by Level 1 studies. Although the BRAVA® device is innovative as a preliminary prototype, significant developments in terms of improved comfort of wear during the night, more reliably maintained seals, and complications related to skin irritation are required to assure its place within the field of breast reconstruction.

Chapter 6. Magnetic resonance imaging vs. three-dimensional laser scanning for breast volume assessment following breast reconstruction*

*A condensed version of this chapter was published: HOWES, B. H., WATSON, D. I., FOSH, B., KLEINIG P., YIP J.M., & DEAN, N. R. 2016. MRI vs. 3D laser scanning for breast volume assessment following breast reconstruction. Ann Plast Surg, Vol 78 (4), 2017, 455-459.

6.1 Introduction

Non-contrast Magnetic Resonance Imaging (MRI) has been reported to be a highly accurate method for measuring breast volume, and is considered the method of choice for assessment of silicone implant volume changes (Tepper et al., 2008, Henseler et al., 2014). However, MRI is expensive and requires both a radiographer for image capture and a radiologist to interpret the results. Alternative computer-analysed breast measurement methods include digital mammography, biometric analysis and 3D photography which have been demonstrated to be non-specific for breast volume measurement[8]. The 3D whole body laser scanner developed by Cyberware has specially developed Cyslice software by Headus. Our department has previously validated this technique against water displacement of mastectomy specimens, and shown an excellent correlation between 3D laser scanning and displacement techniques (Yip J.M, 2012). However, there have been no direct studies comparing non-contrast MRI with actual mastectomy specimen volume. To address this we evaluated the relationship between non-contrast MRI and the previously validated 3D laser scanning technique.

6.2 Methods

6.2.1 Ethics Approval Number

The Ethics committee approved a study into the use of this system. (Southern Adelaide Clinical Research Ethics Committee approval number 321.13)

6.2.2 Aim and hypothesis

The aim of this study was to compare two techniques (3D laser scanning and non-contrast MRI) for measuring breast volume in women undergoing breast reconstruction using autologous fat graft to evaluate equivalence and to identify any potential for bias with either of these modalities. The study hypothesis was that women who undergo breast MRI to assess breast volume will have comparable results using a 3D laser scanner.

6.2.3 Recruitment and scanning

A prospective method-comparison cohort study was undertaken in a group of women who had previously undergone either breast conserving surgery or total mastectomy and who had then attended the Breast Reconstruction Service at Flinders Medical Centre (Adelaide, South Australia). Women were recruited prospectively once they were consented for an external expansion method BRAVA© and autologous fat grafting procedure (Khouri R.K., 2012). Scans were taken after external expansion with the BRAVA device and the day before autologous fat grafting, and then 6 months post-operatively. Scans were taken of the treated breasts (breast conserving surgery or total mastectomy) and normal contralateral breasts. To compare both modalities, scans were taken one after the other, as near as possible to simultaneous analysis. Women underwent non-contrast breast MRI in the radiology department and then attended the Medical Illustration and Media unit for a 3D laser scan. Three-dimensional (3D) laser scans were performed using a Cyberware whole-body laser scanner and Cyslice software (Headus, Australia) was used to calculate the volume of the breasts. This was done using a protocol previously described at our institution in the validation study by et al. *(Yip J.M, 2012)*. The volume of the breast at MRI scan was determined by the following equation; Anteroposterior x superior to inferior x medial to lateral diameters/2, which is the equation used to determine the volume of an elliptical cone (Monticciolo et al., 1994). The strength of the MRI was 1.5 Tesla with 20mT/m, and bilateral dedicated breast surface multichannel coils were used. To reduce the potential for inter-rater variability, the same investigator undertook both pre and post-operative 3D laser scans. Similarly, the same radiologist undertook all the breast MRI calculations. Standardized photographs were used to correlate symmetry and confirm volume differences seen on 3D scanning.

6.2.4 Statistical Analysis

Normal distribution of the numeric variables was assessed by the Kolmogorov–Smirnov test. A paired t-test was used to establish any statistical difference in the mean between the two tests at a significance level of $P<0.05$. All statistical calculations were performed using SPSS v22.0 software (IBM Corp, Armonk NY, 2013). To assess agreement between the two measures a Bland Altman plot was used to determine relationships between the magnitude of bias and any degree of variation (Bland and Altman, 1986). Given the study hypothesis of no difference between the two measures, a One-Sample t Test was used to determine mean difference between the two measures. The standard deviation was multiplied by 1.96 and added to the mean difference to establish the confidence interval for the upper limit score. For the lower limit score the standard deviation was multiplied by 1.96 but deducted from the mean difference. If 0 is perfect agreement, 95% in limits of agreement were used to determine bias. To evaluate reproducibility a coefficient of variation (CV= 100 x SD/mean) was calculated expressed as a percentage of mean breast volume.

6.3 Results

Eighteen patients were scanned pre and post operatively. Scanning at two time points yielded 72 3D breast volume scan measurements and 72 MRI measurements for comparison. Although the 3D scan on average identified smaller volumes, the one-sample t-test established no significant difference between non-contrast MRI and 3D laser scan samples (p= 0.354, 95% CI = -47.84 to 17.35, mean difference -15.24) and therefore the two measurement methods were in agreement. A Bland-Altman plot is shown in Figure 1. and demonstrates no proportional bias between assessment methods. Linear regression analysis was used to confirm a lack of proportional bias (mmean = - 0.107, beta = - 0.216, t = -1.79, p = 0.078). The linear regression t test was not significant and there was no proportional bias. There was no trend for more points to be above or below the mean difference, further supporting the hypothesis that both tests are in agreement.

The Pearson correlation shown in Figure 2 demonstrates a strong linear correlation between the two test methods (r = 0.889, p <0.001). The Co-efficient of variation was (CV= 100 x SD/mean) 66.1% for MRI and 61.8% for the 3D laser scanner.

Figure 6-1 Bland-Altman Plot showing difference in mean volume vs mean volume of scans (MRI & 3D Scan).

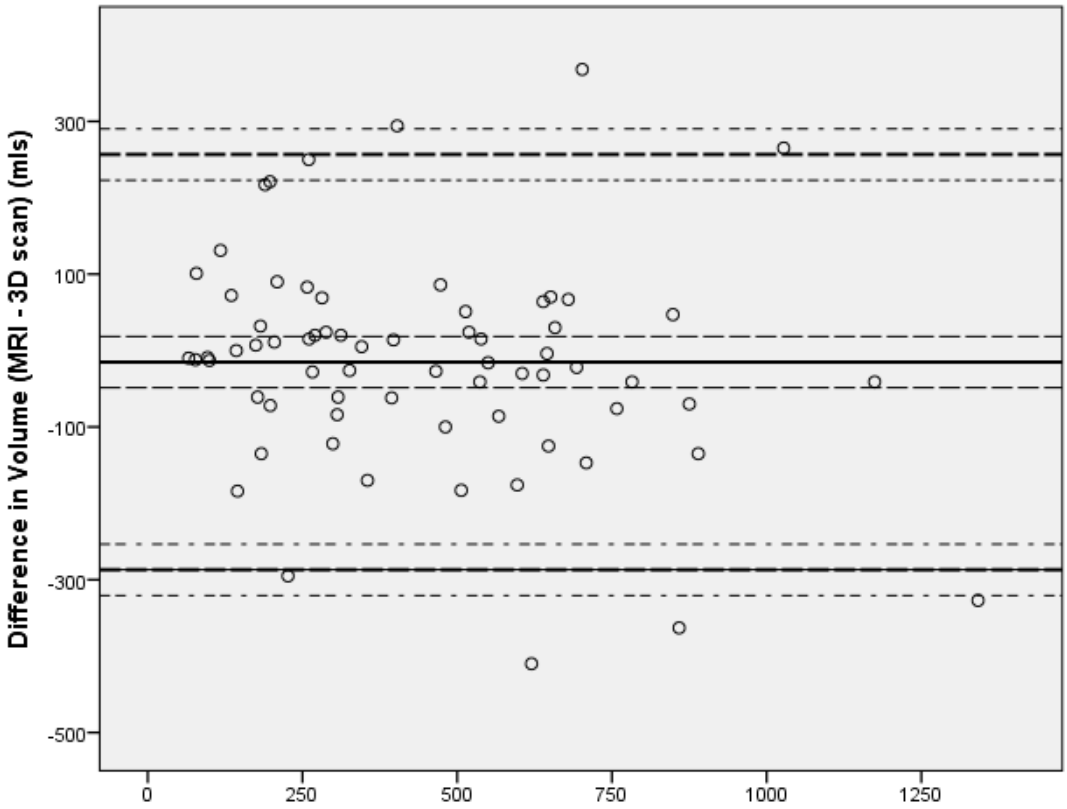
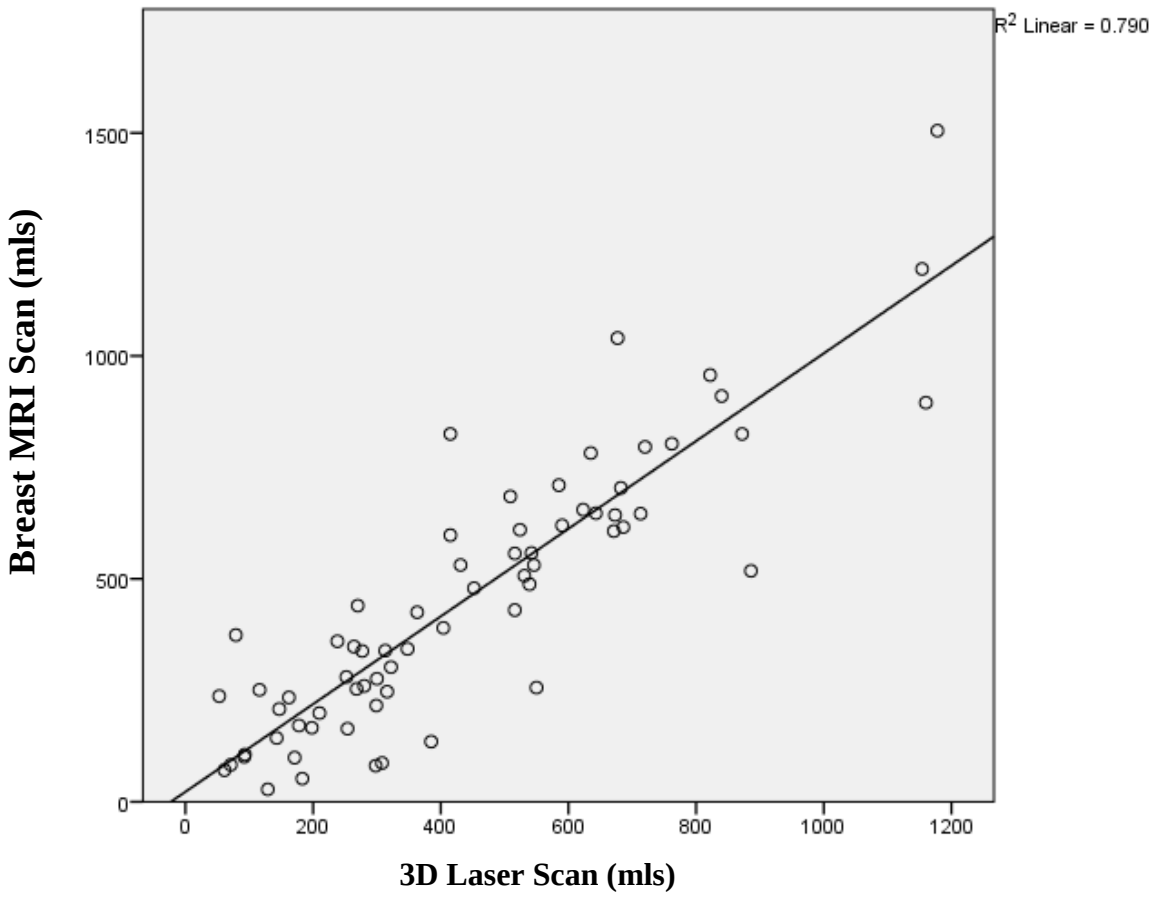


Figure 6-2 Pearson Correlation Breast MRI Scan versus 3D Laser Scan Breast Volumes (p<0.001)



6.4 Case study 1

The following case demonstrates the similar scan volumes from both MRI and 3D scans volumes. The BREAST – Q patient reported outcome measure was used to assess her pre and posoperative change in quality of life. Mrs. LS is a 59-year-old female who previously underwent RoFA mastectomy having completed her breast cancer management and deemed low risk for breast cancer recurrence. She presented to the plastic surgeon for consideration of breast reconstruction. On examination, she was noted to have asymmetry with a ptotic breast on the right and a supple skin envelope after RoFA mastectomy. The patient was not willing to undergo autologous flap reconstruction, so consented to participate in a pilot study of the BRAVA device with autologous fat grafting. Using both 3D scan and MRI pre-operatively Figure 3 it was determined that her average volume difference between breasts was approximately 230mls and 284ml respectively. This information pre-operatively was used to establish an eventual fat injection amount of 267mls. Scan volumes are outlined in Table 1. Using the scans post-operatively it was determined that the patient had maintenance of fat graft of 32mls, approximately 11.9%. Despite minimal gain with her initial fat grafting procedure, her BREAST-Q improved Table 2, and she was willing to undergo repeat procedures to achieve improved symmetry.

Figure 6-3 Pre Autologous Fat Grafting 3D and MRI images

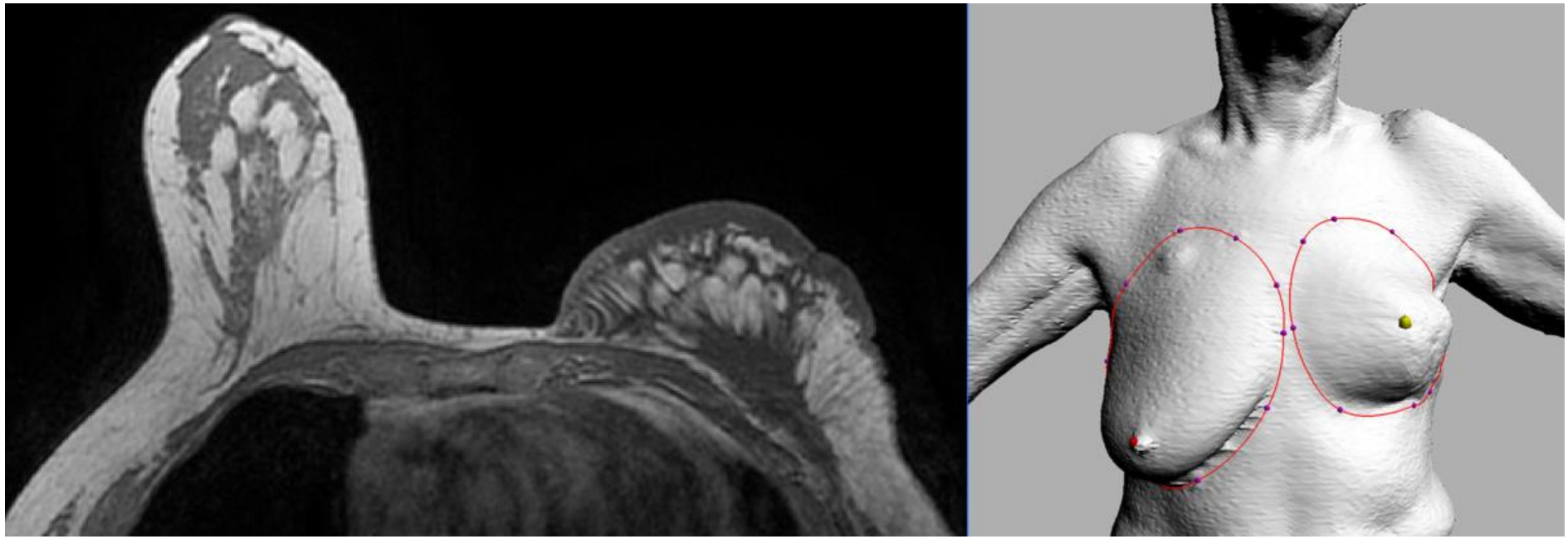


Table 6-1Volume of scan types 3D and MRI pre versus post-operatively

Scan Type	Pre-operative Right	Pre-operative Left	Post-operative Right	Post- operative Left
3D	546	316	542	348
MRI	531	247	558	343

Figure 6-4Post autologous fat grafting images

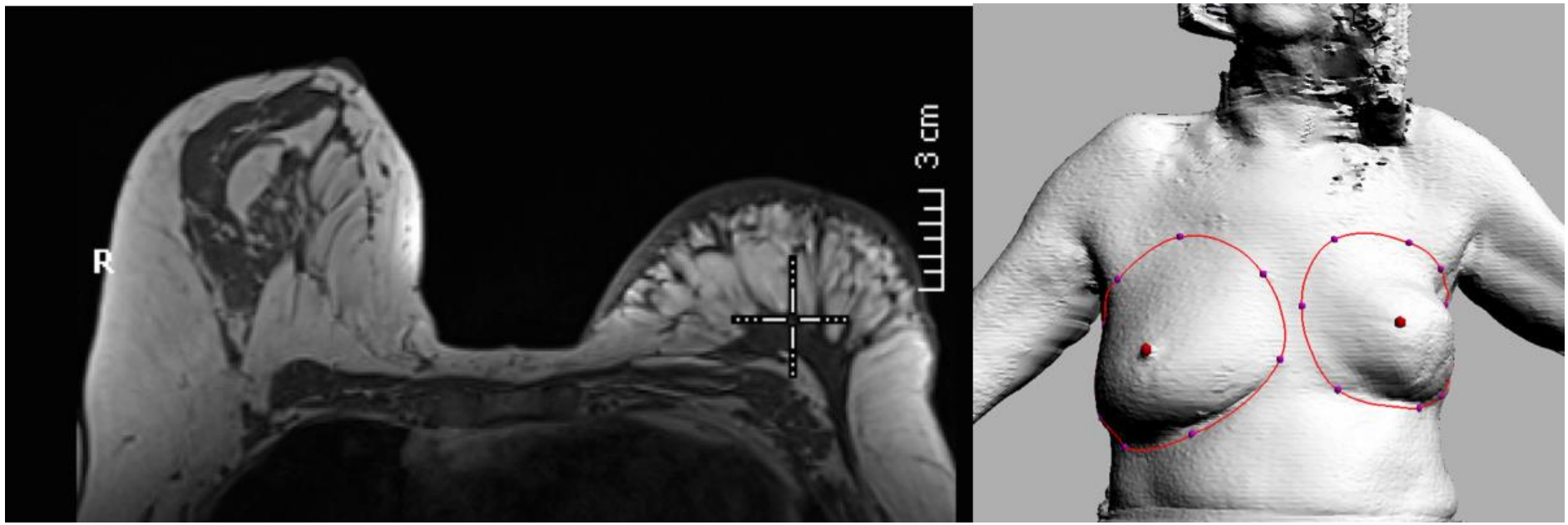


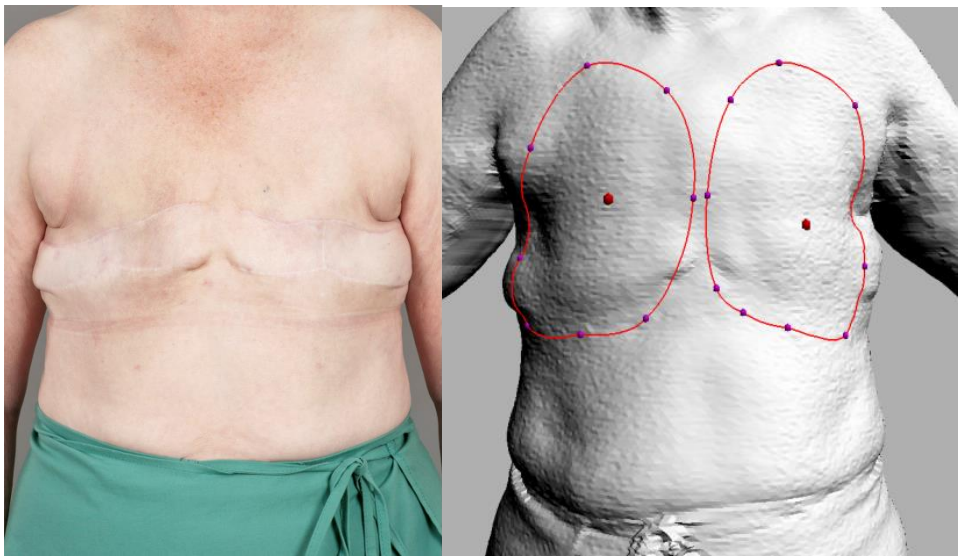
Table 6-2 BREAST-Q scores pre versus post- operatively.

BREAST-Q Domain Score	Satisfaction with Breast	Psychosocial Well-being	Physical Well-Being Chest	Physical Well-Being Abdomen	Sexual Well-being
Pre-Operative	70	82	91	100	83
Post-Operative	73	92	85	90	75

6.5 Case study 2

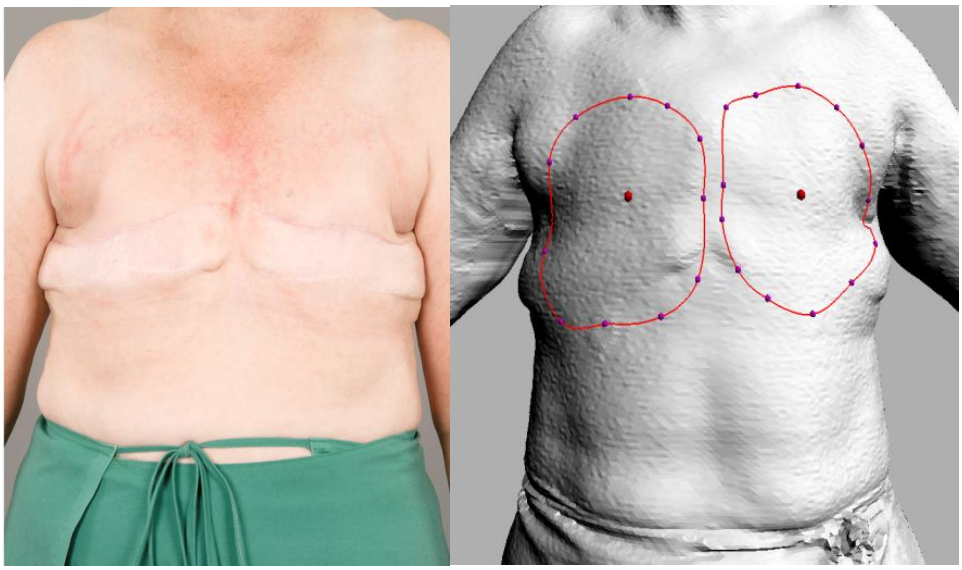
The following case demonstrates the similarity of scan volumes generated from both MRI and 3D scanning. The BREAST – Q patient reported outcome measure was used to assess the patient's pre and post-operative change in quality of life. Mrs. CC is a 64-year-old female who previously underwent bilateral mastectomy with adjuvant radiotherapy. Having completed her breast cancer management and deemed low risk for breast cancer recurrence, she presented to the plastic surgeon for consideration of breast reconstruction. On examination, she was noted to have horizontal scars from her bilateral mastectomy and no overt radiotherapy damage to her breast tissue. The patient was not willing to undergo autologous flap reconstruction, so she consented to participate in a pilot study of the BRAVA device with autologous fat grafting. Using both 3D scan and MRI pre-operatively Figure 5. it was determined that her average volume difference between breasts was approximately 23mls and 13mls respectively. This information pre-operatively was used to establish an eventual fat injection amount to improve symmetry of 189mls to the right and 200mls to the left side. Using the scans post-operatively Figure 6. the amount of residual graft was estimated based on the injected volume. Pre-operative radiotherapy may have explained her poor clinical outcome and it is noted in her BREAST-Q results Table 3. that she had decreased satisfaction post procedure. Understandably, she was unwilling to undergo further fat grafting procedures after being advised that she would require further procedures to achieve the outcome she would find satisfactory.

Figure 6-5 Pre AFG MRI vs 3D scan volumes



Scan Modality Pre-Op	Right	Left
MRI	74	51
3D	79	66

Figure 6-6 Post AFG MRI vs 3D scan volumes



Scan Modality Post-Op	Right	Residual Graft and Proportion Right (189mls)	Left	Residual Graft and Proportion Left (200mls)
MRI	83	9mls (4.7%)	71	20mls (10%)
3D	71	8mls (4.2%)	61	5mls (2.5%)

Table 6-3 BREAST-Q scores pre versus post- operatively.

BREAST-Q Domain Score	Satisfaction with Breast	Psychosocial Well-being	Physical Well-Being Chest	Physical Well-Being Abdomen	Sexual Well-being
Pre-Operative	53	86	71	100	72
Post-Operative	28	84	78	100	0

6.6 Discussion

Magnetic Resonance Imaging has been demonstrated to be highly sensitive and specific for the detection of breast cancer, as well as monitoring the efficacy of neoadjuvant therapies (Marinovich et al., 2015). In younger women with dense breasts, it is easier to identify breast lesions than mammography. It has also been referred to as the gold standard for checking integrity of silicone implants following rupture (Khouri R.K., 2012). Its use in breast reconstruction for breast volume measurement is limited in the literature with a few publications including only small cohorts of patients. Kovacs et al. study in 6 patients identified slightly better precision with MRI versus a 3D scanner (Kovacs et al., 2007). To validate the 3D laser scanner used at our institution, we previously used Archimedes principle of water displacement for mastectomy specimens , and found the 3D scanner to be highly accurate (Yip J.M, 2012). The current study represents a separate validation against the more widely accepted MRI technique.

3D laser scanning was first created in engineering for spatial mapping in the 1960's(MAB, 2011). Cyberware Inc. (Monterey, California)(MAB, 2011) designed a 3D laser scanner (WBX model) which was used in a large anthropometric study called CAESER in 1998 to design uniforms for the military (Robinette, 2002). Later it was used in animation for mapping of the surface anatomy of fish for the movie "Finding Nemo" in 2003 (R, 2008). Soon thereafter the application was used in humans and demonstrated usefulness in the clinical field and breast reconstruction (Yip et al., 2015, Yip J.M, 2012, Bulstrode et al., 2001, Kovacs L, 2005, Kovacs et al., 2006, Kovacs et al., 2007, Tepper et al., 2009, Tzou et al., 2014, Choi et al., 2013, de Heras Ciechomski et al., 2012). The first whole body laser scanner designed by Cyberware in the 1990s used this principle in its design with four

cameras set at a known focal length which project a laser to collect data points in space on a subject and subsequently generate a 3D mesh using compiling software. Laser measurements can be exact within a millimetre using the principal of triangulation. However, high quality validation studies are lacking; particularly for the purpose of peri-operative planning in breast reconstruction. Bulstrode et al. compared five different techniques: MRI, thermoplastic moulding, anatomical measurements, Archimedes principle and mammograph (Bulstrode et al., 2001). Unfortunately, they were unable to demonstrate equivalence between modalities, partly due to the small sample size. The scanner used in the current study has served to assist in complex breast reconstruction cases (Yip J.M, 2012). In one particular case, the scan measurements proved very useful in a patient undergoing whole breast reconstruction using autologous fat graft on the right side and simultaneous reduction of the left breast (Howes et al., 2014). The use of the 3D scanner pre-operatively helped to determine the amount of fat graft harvest for reconstruction including an overcorrection of 30% to account for reabsorption, and the amount which would need to be reduced in order to achieve symmetry. Post-operatively the 3D laser scanner was used to determine the fat graft retention rate.

Highly accurate measurement methods are required in the increasingly complex field of breast reconstruction where success of techniques such as autologous fat grafting can only be tested if quantitative measurement is available. Losken et al. (Losken A., 2005) previously validated 3D scanning technology demonstrating its usefulness our institution has used 3D laser scanning for breast reconstruction for the past 5 years and has been found to be useful in assessment of symmetry (Yip et al., 2015).

Nahabedian et al. reviewed 334 women who had undergone breast reconstruction over a 4 year period (Nahabedian and Galdino, 2003). Thirty-three of these patients had 3D digital photography and this group were compared with the remaining 301 un-scanned patients (Nahabedian and Galdino, 2003). The groups of patients they compared using this technology were women who had undergone autologous flaps versus two stage implant reconstruction. This study concluded that 3D imaging was most useful in identifying asymmetry after primary reconstruction. We consider 3D imaging to have several more applications relating to breast reconstruction: identifying volume asymmetry after mastectomy, measuring pre versus post breast reduction volumes, assessing fat graft retention rates following autologous fat grafting, estimating expander volumes in patients' from other centres, and planning for symmeterisation after autologous flap or implant-based reconstruction. The case in this paper demonstrated the usefulness of evaluating patients who are undergoing autologous fat grafting. The patient was able to have measurement of degree of breast asymmetry; Amount of fat grafting which would be required to improve breast symmetry and then the amount of fat reabsorption measured over time.

The average cost of a breast MRI at our centre is approximately US$500 per scan and the initial cost of an MRI machine depends on the type; 1.5 T versus 3T. Caruso et al. specified costs for time and material of US$1400 for MRI(Caruso et al., 2006). Comparison with other modalities (6.6) demonstrates new technologies that have not yet been validated and vary considerably in initial outlay cost (Bulstrode et al., 2001), ranging from US$20,200 - 76,000 (Kovacs et al., 2006). Initial set up fee, ongoing maintenance cost and software licensing also depends on the provider (Tepper et al., 2009). After purchasing the 3D laser scanner there are cost

considerations for infrastructure for maintenance, room rental, electricity, and the cost of personnel to run the scanner and image analysis. However, the majority of scans taken at our institution were done by a breast reconstruction clinical nurse practitioner, and analysis was done using software which does not have ongoing software licensing costs. Hence, cost for 3D scanning is likely to be cheaper than MRI. 3D scanners are not yet common place in breast surgery owing to the initial outlay of setting up a 3D scanner which can be relatively high. In some countries, there may also be questions of insurance reimbursement for 3D analysis which would need to be finalised before this technology is used on a larger scale. However, 3D scanners are becoming cheaper and more readily available and its likely their use will increase in years to come.

The time for breast MRI scan without gadolinium is equal to the time of a 3D laser scanner, however, there are usually less time pressures with the use of a 3D scanner and it is more efficient regarding timing of patients' appointments for breast volume measurement. By demonstration of practicality and reproducibility we have demonstrated that 3D laser scanner overcomes issues previously considered limitations of 3D surface imaging for volume measurement (R, 2008). After familiarisation with the software for breast volume analysis, each subsequent analysis can be done in approximately 10 minutes, this too refutes earlier claims regarding difficult post scan analysis (Yip J.M, 2012).

The Canfield Vectra system with Geomagic software (Canfield Scientific Inc., Fairfield NJ, USA) was used in the study by Choi et al. investigating fat graft survival rates after breast reconstruction and although graft retention rates correlate with clinical expectations of 27.1 – 52.3% (Choi et al., 2013). Fat graft retention rates of only 20% have been reported by other authors with an inverse correlation of

survival to injection volume (Bulstrode et al., 2001). The retention rates of autologous fat grafting in women who have undergone breast reconstruction post breast cancer surgery is an area of ongoing investigation. The Vectra system, used in the study by Choi et al., has not been validated (Choi et al., 2013). So that the scanner can be fitted into an office space the machines are breast only machines rather than whole body scanners and there are additional machines advertised for purchase separately which are also able to scan the face. A 3D laser scanner (Crisalix 3D MAMMO simulator, Lausanne, Switzerland) was used to scan 4 post-operative patients by de Heras Ciechomski et al. (de Heras Ciechomski et al., 2012) This data was then compared retrospectively with 3D images created from 2D photographs of patients using graphics software Unity3D. Given the mixed design of this study, low sample size and lack of a gold standard modality for comparison, it is difficult to draw meaningful conclusions regarding system validation.

Although both MRI and 3D scans are accurate, both have their limitations. Women are required to lie prone in the MRI scanner coils which makes the breast tissue susceptible to gravity, and this increases the anteroposterior diameter of the breast. The equation for the volume of an elliptical cone is an approximation and overall volume increases above the true breast volume when the breast elongates. Measuring three planes may also be a limitation for MRI given that it does not consider breast contour and the concavity of the chest wall. MRI takes internal breast plane measurements whereas the 3D laser scanner measures the skin surface. For this reason, Yip et al. calculated a correction factor, used to 'subtract' the skin from volume improving accuracy (Yip et al., 2015). Tepper et al. divided 3D images into 3 planes to calculate breast volume in 14 patients (Tepper et al., 2008). Although this method is similar to what was used in our study to measure breast MRI volumes they

did not yield significant increases in breast volume measurements after an implant was inserted which may be related to the small sample size in their study. Later the same authors published a series of 30 patients' pre and post breast reduction which outlined the utility of using a 3D scanner and Geomagic software to observe changes in breast volume and anteroposterior projection of the breast (Tepper et al., 2010). Another difficulty with MRI measurement has been determination of the caudal-cephalic measurement. This is because it is difficult to distinguish the infra-mammary fold from the subcutaneous fat of the abdomen. These reasons may explain why the coefficient of variation was slightly better in the 3D scanner, although the coefficient of variation results confirmed similar variability in the data measures.

6.7 Conclusion

This current study suggests 3D laser scanning with an established protocol is equivalent to non-contrast MRI for the assessment of breast volume. Given the likely lower cost and convenience of laser scanning compared to MRI, this is relevant for plastic surgeons performing complex breast reconstruction work.

Chapter 7. *In vitro* behaviour of breast cells in Autologous Fat Graft environments.

7.1 Introduction

Currently, there is controversy surrounding the use of lipoaspirated fat in women with a history of breast cancer. The *in vitro* studies designed to mimic autologous fat grafting show conflicting information regarding the promotion of oncogenesis (Krastev et al., 2013, Krumboeck et al., 2013), and due to Petit's clinical studies demonstrating a higher cancer recurrence rate in women with a history of ductal carcinoma *in situ* who have undergone fat transfer, it is pertinent to explore this issue further (Petit et al., 2012).

Two-dimensional models involving autologous fat and breast cells have been an area of interest when observing appropriate treatments for breast cancer adjuvant therapies. Three-dimensional models have been used to validate the finding of two-dimensional studies as they closely represent the environment of in vivo and in situ environments. There are few studies which have explored the relationship between autologous fat and breast cells despite the current controversy in the clinical literature. The purpose of this chapter was to attempt to define this relationship pre-clinically using both two-dimensional and three-dimensional cell culture models.

7.2 Aim

To improve understanding of whether autologous fat grafting increases the risk of cancer recurrence in women with previous breast cancer or ductal carcinoma in situ, we sought to investigate the relationship between breast cells in different culture microenvironments.

7.2.1 Specific aims:

1. To establish whether fat transfer creates a microenvironment that predisposes benign breast cells to behave abnormally;

2. To establish whether cancer cells proliferate more rapidly in the presence of autologous fat than in baseline conditions;

3. To establish whether conditioned media derived from different fat sources has different effects on both a normal cell line (MCF10a) and a breast cancer cell line (MCF7):

    a. media conditioned with lipoaspirate from an abdominal fat harvest

    b. media conditioned with fat derived from previously transferred fat (BRAVA patients who underwent re-grafting);

    c. media conditioned with fat derived from normal subcutaneous fat e.g. abdominoplasty;

    d. media conditioned with fat derived from autologous flaps (TRAM, LD, or revision patients).

4. To establish whether whole adipocytes from different sources have different effects on both a normal cell line (MCF10a) and a breast cancer cell line (MCF7) in co-culture:

a. adipocytes aspirated from the abdomen during fat grafting procedures;

b. adipocytes derived from previously transferred fat (BRAVA patients who underwent re-grafting);

c. adipocytes derived from normal subcutaneous fat e.g. abdominoplasty;

d. adipocytes derived from flaps (TRAM, LD, or revision patients).

7.3 Materials and Methods

7.3.1 Ethics Approval

The Ethics committee approved the study entitled *Co-culture of adipocytes with MCF10a and MCF7 cells in two-dimensional and three-dimensional microenvironments* (Southern Adelaide Clinical Research Ethics Committee approval number 355.14). All patients read participant information sheets prior to signing consent forms for the research and the surgical procedure (Appendix 1).

7.3.2 Recruitment

Participants were recruited from eligible women who were undergoing elective procedures at Flinders Medical Centre (FMC) which included autologous fat grafting for breast reconstruction, abdominoplasty and nipple reconstruction. The bookings database was used to access the list of eligible patients. Patients were given a participant information sheet about the study prior to giving consent.

Patients who expressed interest in participating in the study were given an opportunity to discuss any queries they had after reviewing the participant information sheet, and were enrolled in the trial by one of the investigators after checking that they met inclusion and exclusion criteria and obtaining written consent. A study-specific consent form was used for enrolment along with the standard consent form required for elective operative procedures.

7.3.2.1 Inclusion Criteria

1. Patients who are undergoing autologous fat grafting
2. Patients who have previously undergone autologous fat grafting
3. Patients who are scheduled to undergo elective surgical procedures, including thoracotomy, midline abdominal surgery, and nipple reconstruction

7.3.2.2 Exclusion Criteria

1. Women with untreated breast cancer

7.3.3 Overview of Procedures

Adipose tissue was obtained from all participants and taken to the laboratory where it was digested, yielding both adipocytes and pre-adipocytes. The adipocytes were maintained in a culture to yield conditioned media, which was later placed with MCF10a and MCF7 cells in two-dimensional and three-dimensional models. The two-dimensional conditioned media experiment produced growth curves that were counted using a haemocytometer. Photographs were taken in order to review any morphological changes.

The pre-adipocytes were grown in their own media until confluence, at which point they were frozen for a later date. Once needed for experimentation, the pre-adipocytes were thawed, grown to near confluence, and then differentiated into adipocytes using differentiation media. The adipocytes were then placed with MCF10a and MCF7 cells in two-dimensional and three-dimensional models. For the two-dimensional models, photos were taken to observe any morphological changes. three-dimensional models were assessed using the Operetta High Content Imaging System (PerkinElmer, Waltham, Massachusetts, U.S., www.perkinelmer.com)

7.3.4 MCF10a and MCF7 cells

MCF10a (ATCC® CRL-10317™) and MCF7 (ATCC® HTB-22™) cells were obtained from the American Type Culture Collection (ATCC), 10801 University Boulevard Manassas, VA, 20110, USA.

The MCF10a cells are an immortal breast derived epithelial cell line that is non-tumourigenic and that does not demonstrate terminal differentiation; that is, they are a non-transformed human breast epithelial cell line. In terms of morphology, the MCF10a cells represent a robust cell line to use to investigate changes related to fat graft injection because they maintain many of the characteristics of normal breast cells (Kiosses et al., 2001). Under the confocal microscope, they appear normally as luminal ductal cells with a polygonal cobblestone appearance (Figure 1.) (Kiosses et al., 2001). They form a monolayer when grown on plastic and a ductal structure when cultured within a collagen matrix. The culture media, thawing protocols, cryopreservation, and propagation protocols used in the study were based on what was recommended by the supplier.

The MCF7 cell lines are from a pleural effusion in the setting of mammary adenocarcinoma. They do not have a cobblestone appearance when confluent and stationary (Figures 2. and 3.), which suggests they are constantly motile (Kiosses et al., 2001).

Figure 7-1 MCF10a cells (control) in culture at near confluence (note the 'cobblestone' appearance of stationary cells)

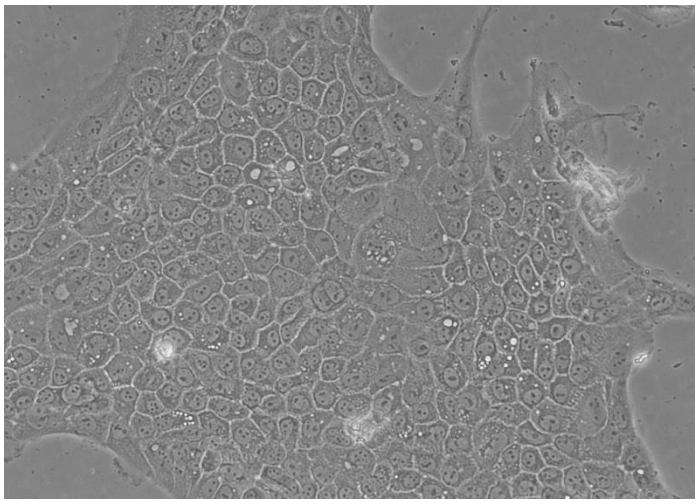
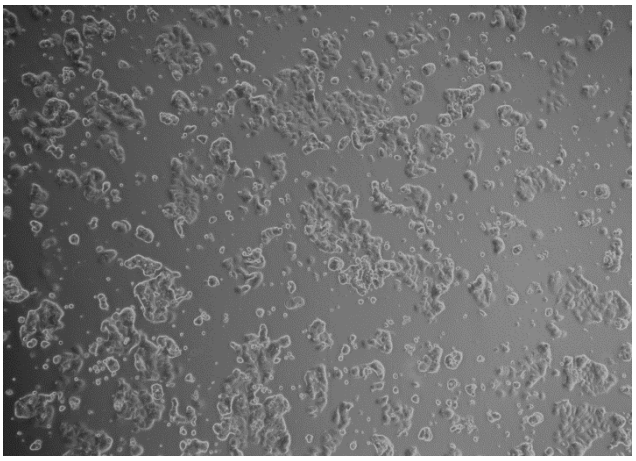
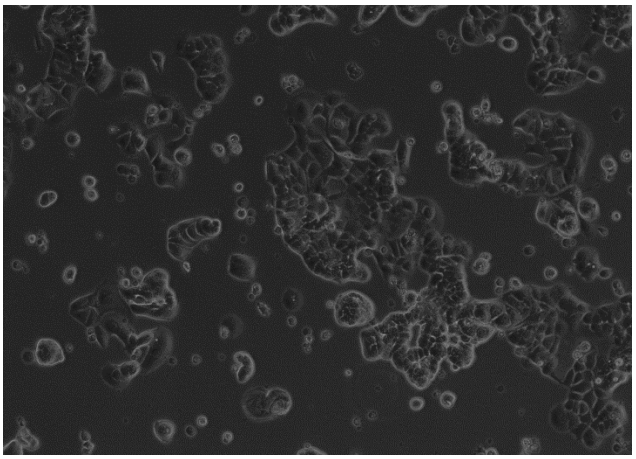


Figure 7-2 MCF7 cells at 60% confluence (10x and 40x magnification)





7.3.5 Culture media and three-dimensional matrix

Six main types of culture media were used: the adipocyte media was mixed as per Lee and Fried (Lee and Fried, 2014), the main ingredients were Dulbecco's Modified Eagles Medium (DMED) with Ham's F12 (1:1 ratio) and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, used as a buffer in cell culture as it resists changes in carbon dioxide maintaining a steady pH) with foetal bovine serum at 5%.

The preadipocyte freezing media was used to freeze preadipocytes and made up as per Lee et.al. (Lee and Fried, 2014). It consists mainly of α-Modified Eagles Media, foetal bovine serum 50% and dimethyl sulfoxide (DMSO). Disposables and other equipment are outlined in Appendix 2. These media are considered the 'standard' media preparation for these cells. When MCF10a cells are placed with conditioned media from the experiments, these were labelled as media from the adipose tissue source from which they were taken (example fat graft).

7.3.5.1 Pre adipocyte and differentiation media

Preadipocyte medium was made up made up as per Yu (Yu et al., 2011), the main ingredients were α-Modified Eagles Media with 10% of foetal bovine serum. In order to differentiate preadipocytes into adipocytes includes human insulin, dexamethasone, rosiglitazone, d-pantothenate, biotin and foetal bovine serum (3%). The recipe is outlined in Appendix 3.

7.3.5.2 The Cultrex® basement membrane

This was used for three-dimensional experiments (Appendix 4). It was obtained from Invitro Technologies® (7-9 Summit Road, Noble Park, Victoria, 3174, PathClear® Catalog #: 3433-010-01) and was supplied to Invitro by Trevigen® (8405 Helgerman Court, Gaithersburg, MD 20877 USA). The Cultrex® growth media and preparation method are outlined in appendix 4. The Cultrex® was used in combination with MCF10a cells in both standard media and media obtained from the fat samples (conditioned media) in order to observe changes in morphology. The preparation of the Cultrex® media and experimentation was carried out as per Debnath et.al. (Debnath et al., 2003).

7.3.5.3 Cultrex® medium (Cultrex® Reduced Growth Factor Basement Membrane Extract)

Cultrex® culture medium was made according to the recipe outlined by the manufacturer.

7.3.6 Adipose tissue harvest

Fat was sampled in an elective setting from predetermined patients who met the inclusion criteria. With pre-operative consent, approximately 5-50 mL of fat was harvested in the following manner:

1. Skin incisions were made using a scalpel and the required volume of adipose tissue was excised using either scalpel or scissors; diathermy excision was not used as this could burn the adipose tissue and render it non-viable. In instances of fat graft harvest (lipoaspirate) intended for injection into the breast, fat that was surplus to requirement for injection into the breast /

mastectomy defect was used for the laboratory study. In some instances, incisions had already been made for the intended procedure (eg. Abdominoplasty operation) and fat was harvested through those incisions.

2. Sterile 50-mL pots were used to transfer the specimens in a plastic bag in cooled water directly to the laboratory.

7.3.7 Adipose Tissue Processing

At the laboratory, the following steps were taken to process the specimens as per Yu et.al.(Yu et al., 2011):

1. Under the lamina flow hood (80% ethanol used for the preparation of all equipment to optimise sterile conditions), the adipose tissue specimens were cut into small pieces, approximately 1–2 mm^3 (5–10 mg) long, using sterile sharp scissors. By using scissors, the crushing of cells was avoided. This was performed using a two-handed scissor technique in plastic 50-cc Falcon tubes.

   (Initially, a McIllwain chopper was used and given the thickness of the feather blade, it was thought that it would not traumatise the adipose tissue; however, the adipocyte yield was less than when scissors were used.)
2. Lipoaspirate specimens were already fragmented and did not require further cutting.
3. Both minced adipose tissue and lipoaspirate specimens were passed through a nylon mesh to filter blood clots. The specimen was washed with saline to remove the local anaesthetic and any other by-products of the operation.
4. After weighing the specimen (20 g to 200 g), 1 mg/mL Collagenase (Type 1) solution per 1 g of adipose tissue was placed in a 50-mL conical Falcon tube.
5. The tops of the tubes were then sealed with paraffin strips, and the tubes were

placed in a 37 °C water bath for 1 hour and agitated every 15 minutes (shaking backwards and forwards).

6. Once the specimens were completely digested, with the cut pieces of adipose tissue having dissolved into micro-droplets, the adipocytes were filtered through nylon mesh to separate the connective tissue.
7. The specimen was washed repeatedly with the culture medium.

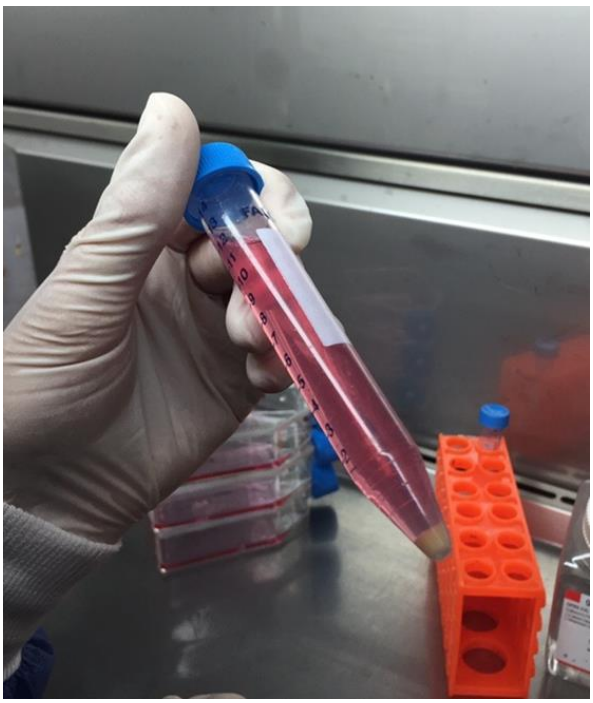
7.3.8 Preparation of conditioned media

In order to harvest the yield of conditioned media obtained from different adipose tissue, the following method was used as described by Wang et. al.(Wang C, 2015) : Specimens were washed and centrifuged at 500 g for a minute. Standard adipose tissue conditioned media was then passed over the top of adipocytes to assist in further separating the specimen into layers and centrifuged for 5 minutes. Oil forms the top layer, followed by adipocytes, the media/red blood cell layer, and adipocyte precursors sediment at the bottom of the tube. The oil layer is carefully aspirated leaving the adipocyte layer exposed, which was then gently aspirated and plated along with adipocyte media. The conditioned media layer was then aspirated with care not to disrupt the adipocyte precursors. Red cell lysis buffer was added to the adipocyte precursor layer for 5 minutes, followed by re-centrifuging and aspirating of red blood cells, which allows for an increased yield of adipocyte precursors (Figures 4 and 5). The adipocyte precursor layer was then plated in media and placed in the incubator at 37 °C.

Conditioned media was obtained daily for the first 3 days (owing to the deep yellow colour that was presumed to be higher concentrations of free fatty acids), and every second day thereafter when the colour of media was more consistent with the rose colour of the Dulbecco's Modified Eagles Medium. Photos were taken to document the progress of the formation of confluent pre-adipocytes that could be either frozen on liquid nitrogen or differentiated into adipocytes

Figure 7-3 Separation into layers: oil, adipocytes, pre-adipocytes, medium

Figure 7-4 Pre-adipocytes



7.3.9 Preparation of pre-adipocytes for co-culture experiments (harvest, subculturing, freezing and thawing)

Pre-adipocyte medium was made up made up as per Yu (Yu et al., 2011) with the main ingredient consisting of α-Modified Eagles Media (Sigma Aldrich, PO Box 970, Castle Hill NSW 1765, Australia http://www.sigmaaldrich.com/australia.html) and 10% foetal bovine serum. Preadipocytes were harvested from each of the fat sample specimens and where subcultured in order to provide higher numbers available for use in experiments. Cells were removed from the incubator and check under the microscope for a 70% confluence of cells. As cells are adherent to the bottom of the flask, Trypsin is used to lift the cells which are then centrifuged in media and counted using a haemocytometer (Figure 6). The cells are then separated into two halves and placed in separate flask for incubation so that the cells re populate each flask in increasing numbers.

The freezing media consists of 50% foetal bovine serum and α-Modified Eagles Media as per Lee et al. (Lee and Fried, 2014). Cells are again Trypsinised and then counted under the haemocytometer. If there were approximately 6 x 10^6 cells, they were divided into one million groups by placing them into 6 separate cryotubes along with the freezing media. These were then placed in -4^{o}C overnight, then liquid nitrogen -196 ^{o}C.

In order to thaw preadipocytes a single cryovial containing 1 x 10^6 was removed from liquid nitrogen and placed into a 37^{o}C water bath for 60-90 seconds or until thawed. These were then re-suspended in pre-adipocyte media and counted for use in each well and incubated overnight so that the cells could bed down on the bottom of the flask. Preadipocytes on day 1 and 4 of growth are shown in Figures 7 and 8.

Figure 7-5 Haemocytometer with MCF10a cells

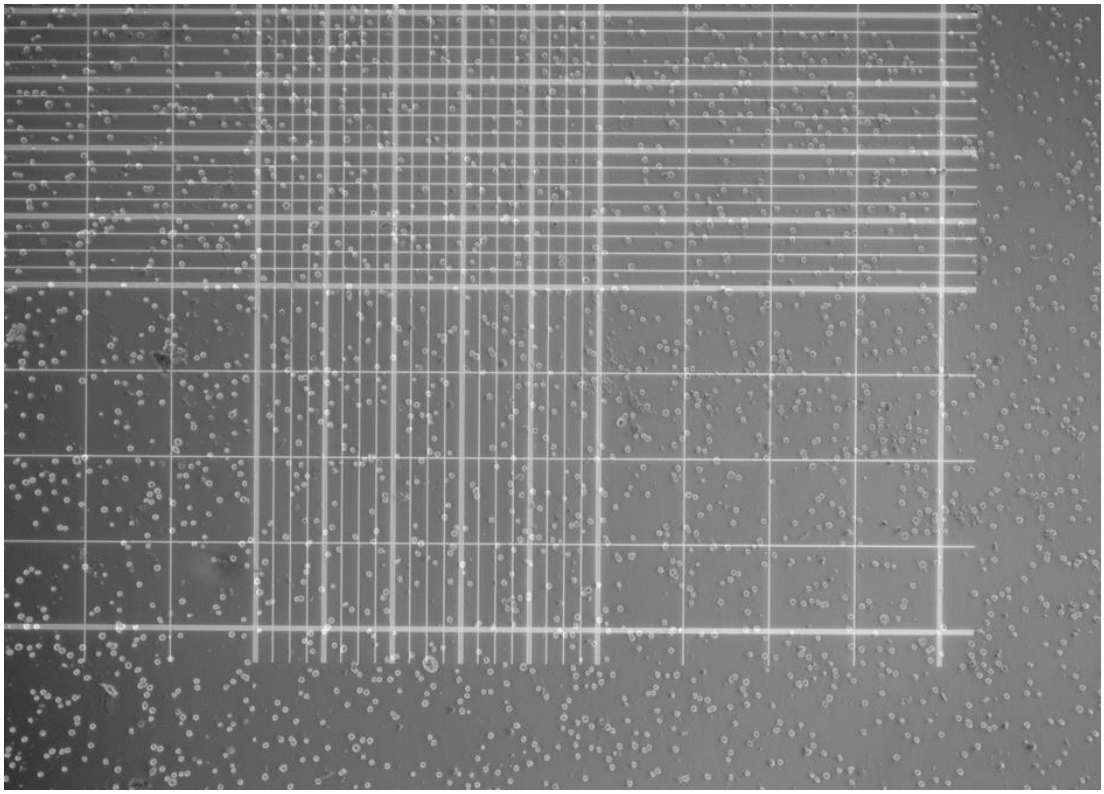


Figure 7-6 Adipocyte harvest and thawing: pre-adipocytes after thawing (day 1 at 40x magnification) and day 4 at 40x magnification.

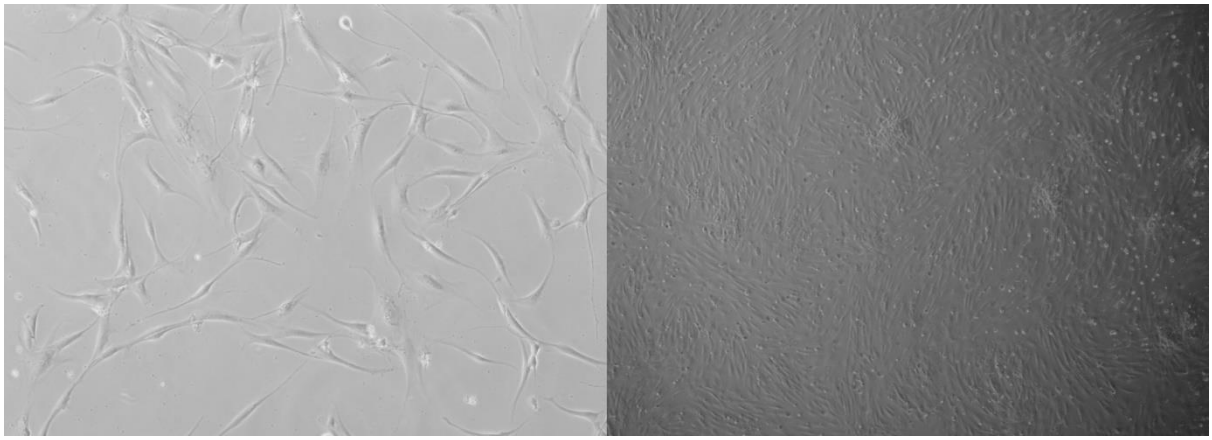
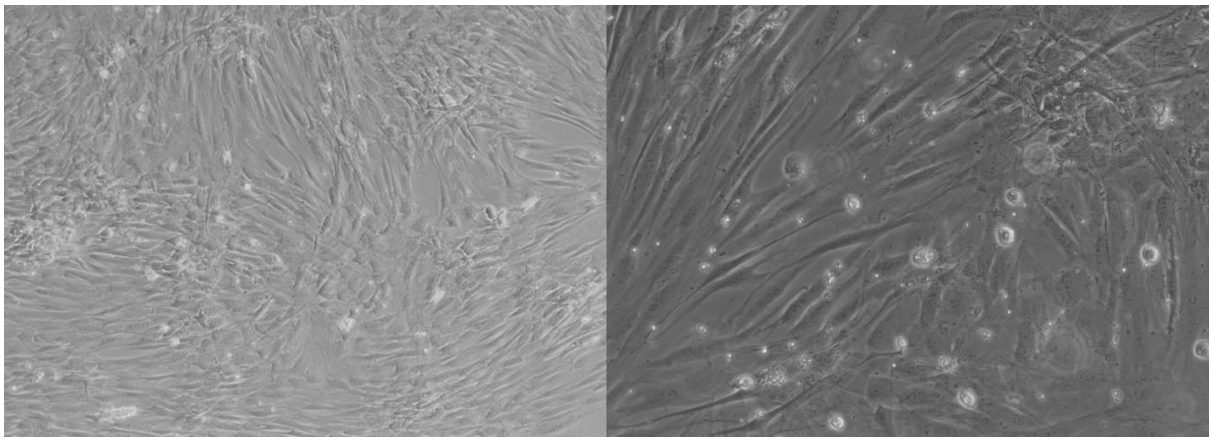


Figure 7-7 Confluent pre-adipocytes (day 4 post thaw) at 10x & 40x magnification



7.3.9.1 Pre-adipoctye differentiation

Pre-adipocytes were grown to confluence in a flask over a couple of days with an approximate plating density of 5,000 cells/cm^2. It took 5-7 days until the cells appeared ready for differentiation. The culture media was then replaced with differentiation media. Differentiation media was changed every 2-3 days. According to Lee and Fried it generally takes up to 12 days for the adipocytes to mature, at which point they can be maintained in the media for up to 40 days (Lee and Fried, 2014). Cells are reviewed under the confocal microscope to observe for lipid formation.

7.3.10 Two dimensional culture of breast cells with conditioned media

7.3.10.1 Two-dimensional MCF10a in conditioned media

MCF10a cells were slowly thawed and allowed to bed down over a 24-hour period prior to adding the conditioned media. Fat-derived conditioned media was mixed in equal parts with the MCF10a media during the co-culturing process. Six thousand (6,000) cells were used per well plate for the groups being treated with conditioned media. A MCF10a cell line was cultured in MCF10a media as a control line. Cells were maintained in the incubator and were counted with a haemocytometer (Figure 6) under the confocal microscope on days 3, 5, and 7 to assess growth rates. Fresh media was replenished in the remaining wells not counted on those days.

7.3.10.2 Two-dimensional MCF7 in conditioned media

MCF7 cells were slowly thawed and allowed to bed down in a 24-well plate, over a 24-hour period prior to adding the conditioned media. Owing to slower doubling time than the MCF10a cell, 8,000 cells were used in each well plate. Fat-derived conditioned media was mixed in equal parts with the MCF7 media during the co-culturing process. An MCF7 cell line was cultured in MCF7 media as a control line. Cells were maintained in the incubator and, along with a control arm, were counted with a haemocytometer under the confocal microscope on days 3, 5, and 7. Fresh media was replenished in the remaining wells not counted on those days.

7.3.11 Three-dimensional culture of breast cells

7.3.11.1 Three-dimensional MCF10a in conditioned media

Cultrex® was thawed overnight and small aliquots were then placed on 24 well plates in a single layer, with care taken not to form bubbles that prevent colony formation, in order to allow the cells to contact the plastic surface. The culture plate was warmed at 37^{o}C for 30 minutes so that the Cultrex® solidifies. After adipocyte isolation (see earlier protocol), approximately 1 x 10^5 MCF10a cells were mixed with Cultrex® and placed on the previous layer of Cultrex® with both the MCF10a media and the Cultrex® basic medium. The medium was changed every 2 days and after 14 days the cells were harvested, wash and analysed. Analysis took place using the Operetta, which measured the size of the nuclei in the three-dimensional matrix.

7.3.11.2 Three-dimensional MCF10a and differentiated adipocytes in co-culture

Steps were conducted as in 7.3.8 except that 1 x 10^6 differentiated adipocytes were combined and incubated along with the 1 x 10^5 MCF10a cells. The MCF10a cells were placed in with the pre-adipocytes that had undergone the process of differentiation. However, under the confocal microscope they had the appearance of MCF10a cells with pre-adipocytes. Further experimentation was not conducted and confocal microscopy photos were taken for academic interest only.

7.3.12 Assessment of adipocyte differentiation- oil red o staining

Cultrex® -containing cultured cells were fixed with 4% formaldehyde, washed in water, and stained with 0.6% Oil red O solution (60% isopropanol, 40% water) for 1 hour at room temperature. Cells were washed extensively to remove un-bound and isopropyl alcohol was added to the stained culture dish. After 1 hour, the absorbance of the extract was assayed by the spectrophotometer at 510 nm.

Although the pre-adipocytes appeared to demonstrate an evolution into an adipocyte shape under the confocal microscope in the differentiation media (Figure 9), it could not be confirmed via Oil red O stain that the pre-adipocytes were in fact adipocytes. Initial explanations include the fact that the Triton-X 100 was needed in order to punch holes into the cells so that the Oil red O could penetrate. However, its addition did not change the outcome. In spite of this, the full period of differentiation was undertaken as per the protocol and the experiments conducted as planned (Figures 10 and 11).

Figure 7-8 Appearance of adipocytes with lipid formation during differentiation

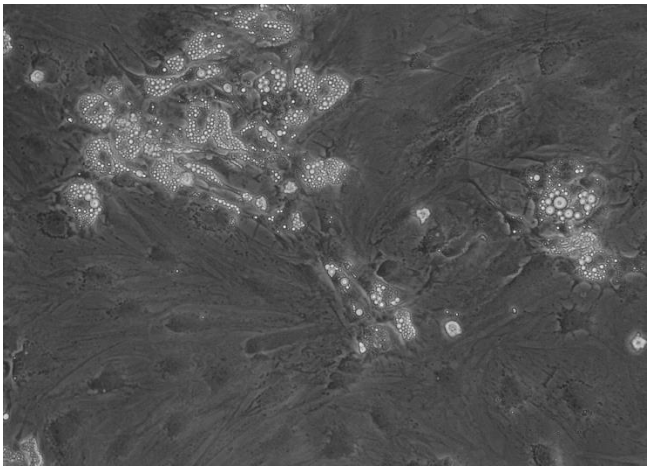


Figure 7-9 MCF10a Cells with fat graft specimen adipocytes (day 10)

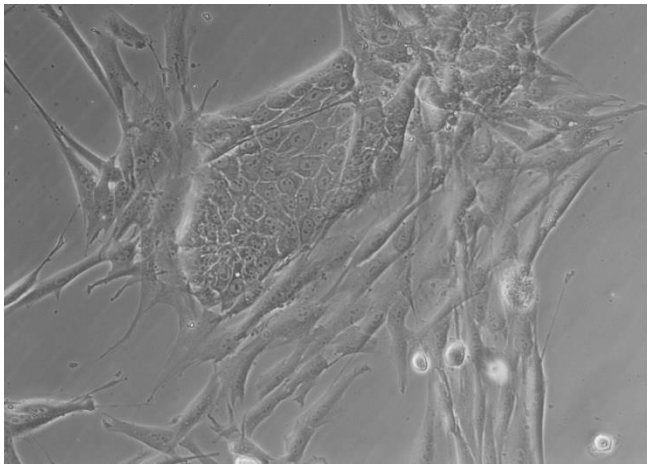
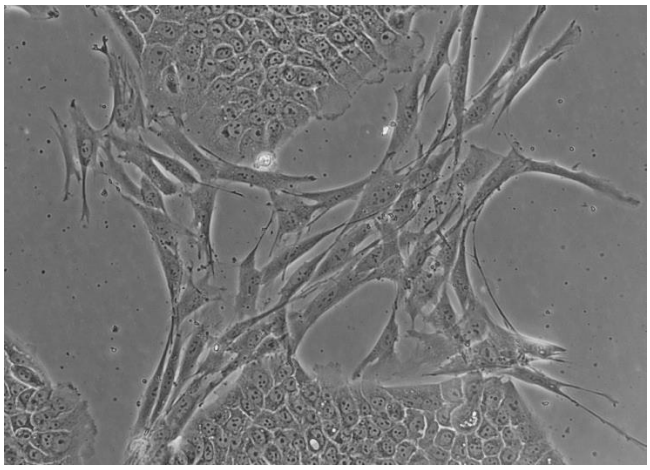


Figure 7-10 MCF10a Cells with previous fat graft specimen adipocytes (day 10)



7.3.13 Assessment of breast cell growth – two-dimensional scratch assay and Incucyte

Plates that were coated with collagen 1 (or another ECM) were incubated overnight. MCF10a cells were placed on top of the thin-layered matrix and allowed to adhere for several hours. A wound area using an Essen 96 Well Wound Maker was made along the centre of the plate. The IncuCyte was then used to automatically collect time-lapse images, observe cell growth, and quantify cell invasion. The Essen Wound Maker and IncuCyte were provided by Essen Bioscience (http://www.essenbioscience.com/en/).

7.3.14 Immunofluorescent staining

The immunofluorescence recipe and procedure are outlined in Appendix 5. Immunofluorescent staining was undertaken for MCF10a cells in 3D culture in order for the Operetta to identify the cell nuclei and measure their surface area and roundedness.

7.3.15 Experimental design and statistical analysis

In order to reduce bias, all experiments were performed with a technical duplicate and a biological triplicate.

1) Technical duplicate: each experiment was repeated twice at the same point in time (i.e. same time and same day).
2) Biological triplicate: the same experiment was performed at three different time points (i.e. different day and different time) in exactly the same manner.

For the growth curves, non-linear regression analysis was undertaken with semi-log values being *x* linear and *y* log using Graphpad Prism 7 software.
(GraphPad Software, Inc.
7825 Fay Avenue, Suite 230 La Jolla, CA 92037 USA). The null hypothesis was that the best-fit values of the selected unshared parameters differed between data sets using the slope of the curve as the identifier. The comparison method was the extra sum-of-squares F test with a p value significance set at $p<0.05$. D'Agostino-Pearson omnibus normality test was conducted confirming that the residuals were Gaussian with appropriate weighting.

7.4 Results

7.4.1 Participants

During the study period, 11 patients were recruited to participate. One participant's fat graft yield (sample 3), from laparotomy tissue, was insufficient for generation of conditioned media. A fat sample obtained from a patient undergoing an abdominoplasty was too small and the subsequent yield of fat preadipocytes was too low, however the conditioned media was used. From that point, it was determined that collection of at least 50 mL of adipose tissue should be attempted for each sample. This left 10 participants with 11 samples.

These were then classified as experiments for the purpose of running tests:

Sample 1: Whole fat specimen (Figure 12) taken during abdominoplasty operation

Sample 2: Whole fat specimen taken during abdominoplasty operation

Sample 3: Whole fat specimen taken during abdominoplasty operation (very low yield: discarded)

Sample 4: Whole fat specimen taken during abdominoplasty operation

Sample 5: Lipoaspirate (Figure 13) taken from the abdomen after harvest using a liposuction cannula (Participant ET)

Sample 6: Lipoaspirate taken from the abdomen after harvest using a liposuction cannula (Participant JW)

Sample 7: Previous breast reconstruction whole fat specimen obtained from a latissimus dorsi flap, (participant undergoing nipple reconstruction procedure)

Sample 8: Previous graft whole fat samples from the breast at the site of previous fat grafting for breast reconstruction following total mastectomy (TS)

Sample 9: Previous graft whole fat samples from the breast at the site of previous fat

grafting for breast reconstruction following total mastectomy (LS)

Sample 10: Previous graft whole fat samples from the breast at the site of previous fat grafting for breast reconstruction following total mastectomy (SK)

Sample 11a: Fat graft whole fat sample from the breast at the site of previous fat grafting

Sample 11b: Whole fat sample from the breast at the site of previous fat grafting

Figure 7-11 Whole fat specimen

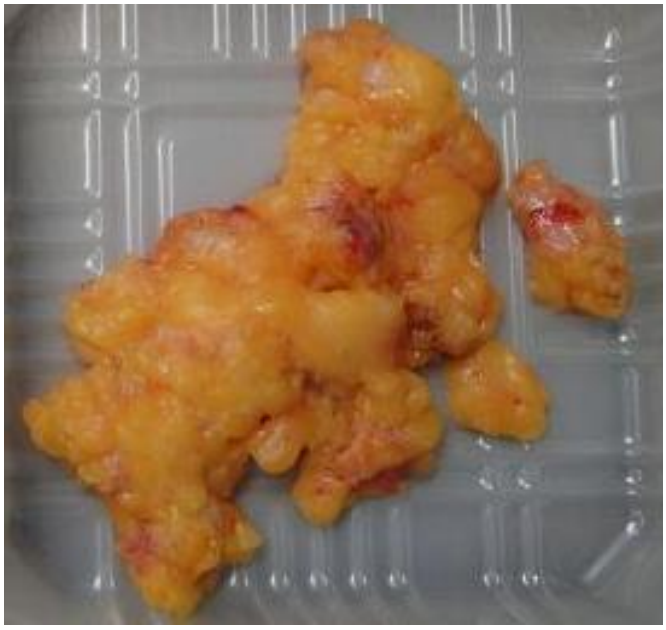


Figure 7-12 Fat harvested from liposuction from the abdomen.

7.4.2 Conditioned Media obtained from participants

Conditioned media was harvested after fat processing using the aforementioned protocols. The yield of the conditioned media, which varied depending on the amount of the fat harvested, is set out below. Although the first day seemed to yield the highest quantity of conditioned media, the quality differed from subsequent days. Initially the conditioned media was a darker yellow-orange colour but as the days progressed, the conditioned media seemed to change in quality appearing more like the rose red colour of the DMEM media. It was thought that in earlier days the free fatty acids may have altered the colour but this was not formally tested. . It was then decided that instead of running several experiments based on different days, the conditioned media from days 1, 5, and 7 would be pooled for each experiment using all variations in colour of the conditioned media. The experiments with similar fat types were also pooled. In other words, the conditioned media of all of the fat grafts was pooled.

Table 7-1 Volume of conditioned media generated by days post processing and experiment number.

Control (MCF10a media only)	MCF10a in standard MCF10a culture media
Whole fat (abdominoplasty)	Sample 1,2, 3, 4 and 11a pooled
Lipoaspirate (fat graft)	Sample 5 & 6 pooled
Whole fat (previous graft site)	Sample 8, 9, 10, 11b pooled
Whole Fat (previous flap reconstruction	Sample 7 pooled*

*Pooled conditioned media was the combination of condition media harvested from day 1, 5 and 7.

Table 7-2 Volume of conditioned media generated categorised by days post-processing and experiment number.

Day	Sample 1 (mls)	Sample 2 (mls)	Sample 4 (mls)	Sample 5 (mls)	Sample 6 (mls)	Sample 7 (mls)	Sample 8 (mls)	Sample 9 (mls)	Sample 10 (mls)	Sample 11a (Lipo)	Sample 11b (Whole)
1	8	10	4	17	11	46	5	5	16	5	18
2						30					
3	5	None	5	56	11	37			3		
5		121	6.5	36	9	25	3	3	7.5	3	10
4				9		22.5					
7	10	27	18	30	8	32	3	3	3	3	8
9		22		26		3					
11		10		11							

Table 7-3 MCF10a cell numbers counted over days in biological triplicate vs type of conditioned media

		Technical duplicate 1									Technical duplicate 2								
		Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3	Bio 1	Bio 1	Bio 1	Bio 1	Bio 1	Bio 1	Bio 1	Bio 1	Bio 1
	Day 0	Day 3	Day 3	Day 3	Day 5	Day 5	Day 5	Day 7	Day 7	Day 7	Day 3	Day 3	Day 3	Day 5	Day 5	Day 5	Day 7	Day 7	Day 7
	MCF10a number of cells (x 10^3)																		
Control (MCF10a media only)	6600	9.0	13.4	13.4	17.8	34.3	34.3	58.5	39.8	39.8	8.3	12.4	12.4	20.0	40.0	40.0	51.8	40.9	40.9
Whole fat (abdominoplasty)	6600	8.8	9.3	9.3	20.4	20.5	20.5	42.3	53.4	53.4	10.0	8.9	8.9	26.6	24.9	24.9	46.4	51.6	51.6
Lipoaspirate (fat graft)	6600	8.2	7.8	8.2	26.0	23.2	23.2	47.8	56.5	56.5	9.0	8.2	7.8	19.8	21.5	21.5	48.2	49.3	49.3
Whole fat (previous graft site)	6600	8.2	11.2	11.2	16.2	19.5	19.5	41.7	41.8	41.8	8.0	10.5	10.5	13.8	19.8	19.8	41.3	45.0	45.0
Whole Fat (previous flap reconstruction	6600	7.0	9.0	12.5	36.0	19.0	35.5	49.0	52.5	50.5	7.0	10.5	13.5	47.5	40.5	25.0	52.0	54.0	49.0

Table 7-4 MCF7 cell numbers counted over days in biological triplicate vs type of conditioned media.

		Technical duplicate 1									Technical duplicate 2								
		Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3	Bio 1	Bio 1	Bio 1	Bio 1	Bio 1	Bio 1	Bio 1	Bio 1	Bio 1
	Day 0	Day 3	Day 3	Day 3	Day 5	Day 5	Day 5	Day 7	Day 7	Day 7	Day 3	Day 3	Day 3	Day 5	Day 5	Day 5	Day 7	Day 7	Day 7
	MCF10a number of cells (x 10^3)																		
Control (MCF10a media only)	8000	13.2	12.2	14.5	32.2	32.5	26.7	52.5	50.5	45.8	15.2	12.9	14.5	31.4	33.4	25.0	50.6	48.8	49.2
Whole fat (abdominoplasty)	8000	15.4	14.6	15.3	25.3	38.8	25.8	54.9	54.4	55.8	22.1	15.0	18.4	25.4	38.5	23.1	61.9	53.6	52.1
Lipoaspirate (fat graft)	8000	16.7	16.8	15.5	29.5	42.2	25.5	59.2	61.0	58.7	14.7	15.0	15.7	21.3	40.7	26.0	60.3	59.2	52.5
Whole fat (previous graft site)	8000	18.7	15.8	13.2	32.8	37.0	33.5	65.0	70.8	71.5	14.5	17.8	13.2	33.5	31.7	41.3	60.5	65.5	71.5
Whole Fat (previous flap reconstruction	8000	14	22.5	15	22.5	43	44	63	54	47	18	19	14.5	22	36	37	55.5	58.5	63

Table 7-5 Average number of MCF10a technical duplicate 1 and 2 growth over day's vs conditioned media groups.

	MCF10a														
	Control (MCF10a media only)			Whole fat (abdominoplasty)			Lipoaspirate (fat graft)			Whole fat (prev graft site)			Whole Fat (previous flap reconstruction		
	MCF10a number of cells (x 10^3)														
Day	Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3
0	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
3	8.65	12.9	12.9	9.4	9.1	9.1	8.6	8	8	8.1	10.85	10.85	7	9.75	13
5	18.9	37.15	37.15	23.5	22.7	22.7	22.9	22.35	22.35	15	19.65	19.65	41.75	29.75	30.25
7	55.15	40.35	40.35	44.35	52.5	52.5	48	52.9	52.9	41.5	43.4	43.4	50.5	53.25	49.75

Table 7-6 Average number of MCF7 technical duplicate 1 and 2 growth over day's vs conditioned media groups.

	MCF7														
	Control (MCF7 media only)			Whole fat (abdominoplasty)			Lipoaspirate (fat graft)			Whole fat (prev graft site)			Whole Fat (previous flap reconstruction		
	MCF7 number of cells (x 10^3)														
Day	Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3	Bio 1	Bio 2	Bio 3
0	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
3	14.2	12.55	14.5	18.75	14.8	16.85	15.7	15.9	15.6	16.6	16.8	13.2	16	20.75	14.75
5	31.8	32.95	25.85	25.35	38.65	24.45	25.4	41.45	25.75	33.15	34.35	37.4	22.25	39.5	40.5
7	51.55	49.65	47.5	58.4	54	53.95	59.75	60.1	55.6	62.75	68.15	71.5	59.25	56.25	55

Figure 7-13 MCF10a growth curve in media vs days being cultured

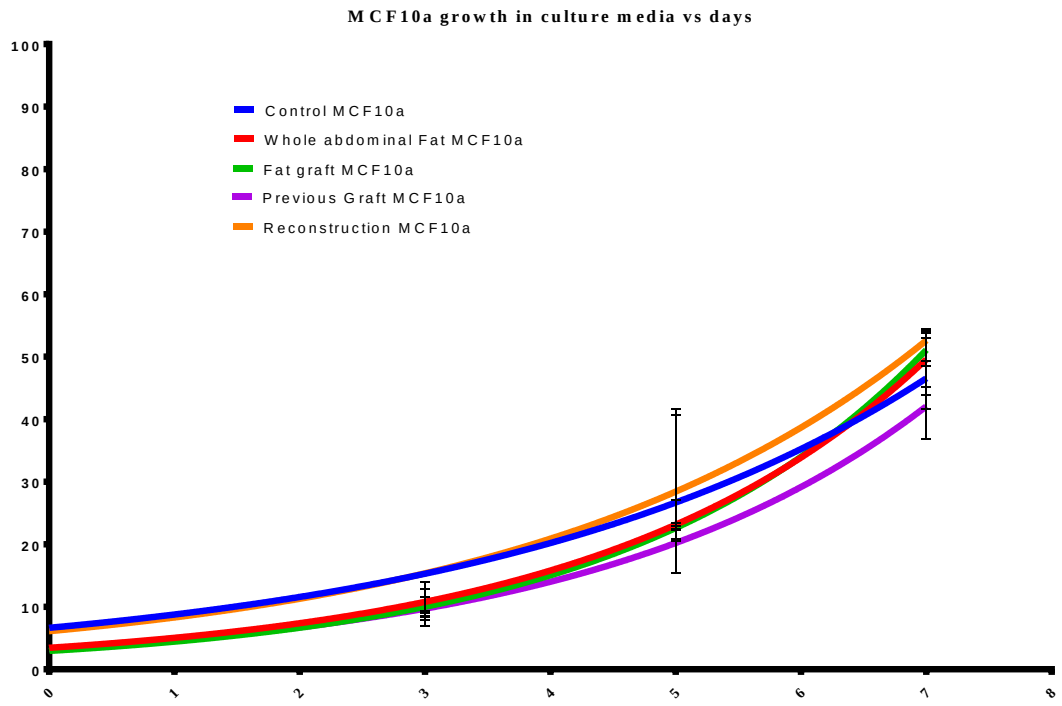


Table 7-7 MCF10a non-linear regression analysis

Curve being compared	Adjusted R square	P value	Confidence interval (Slope)
Control vs Whole abdominal Fat	0.8511 vs 0.9769	0.2741	Control 0.079 to 0.175 Whole abdominal Fat 0.140 to 0.195
Control vs Fat Graft	0.8511 vs 0.9811	0.1433	Control 0.079 to 0.175 Fat Graft 0.1531 to 0.2048
Control vs Previous fat graft	0.8511 vs 0.9679	0.0863	Control 0.079 to 0.175 Previous fat graft 0.1321 to 0.1916
Control vs Reconstruction	0.8511 vs 0.9261	0.4761	Control 0.079 to 0.175 Reconstruction 0.101 to 0.1741
Fat graft vs Previous graft	0.9811 vs 0.9679	0.0013	Fat Graft 0.1531 to 0.2048 Previous fat graft 0.1321 to 0.1916

Figure 7-14 MCF10a in fat graft conditioned media vs time Incucyte scratch assay plot

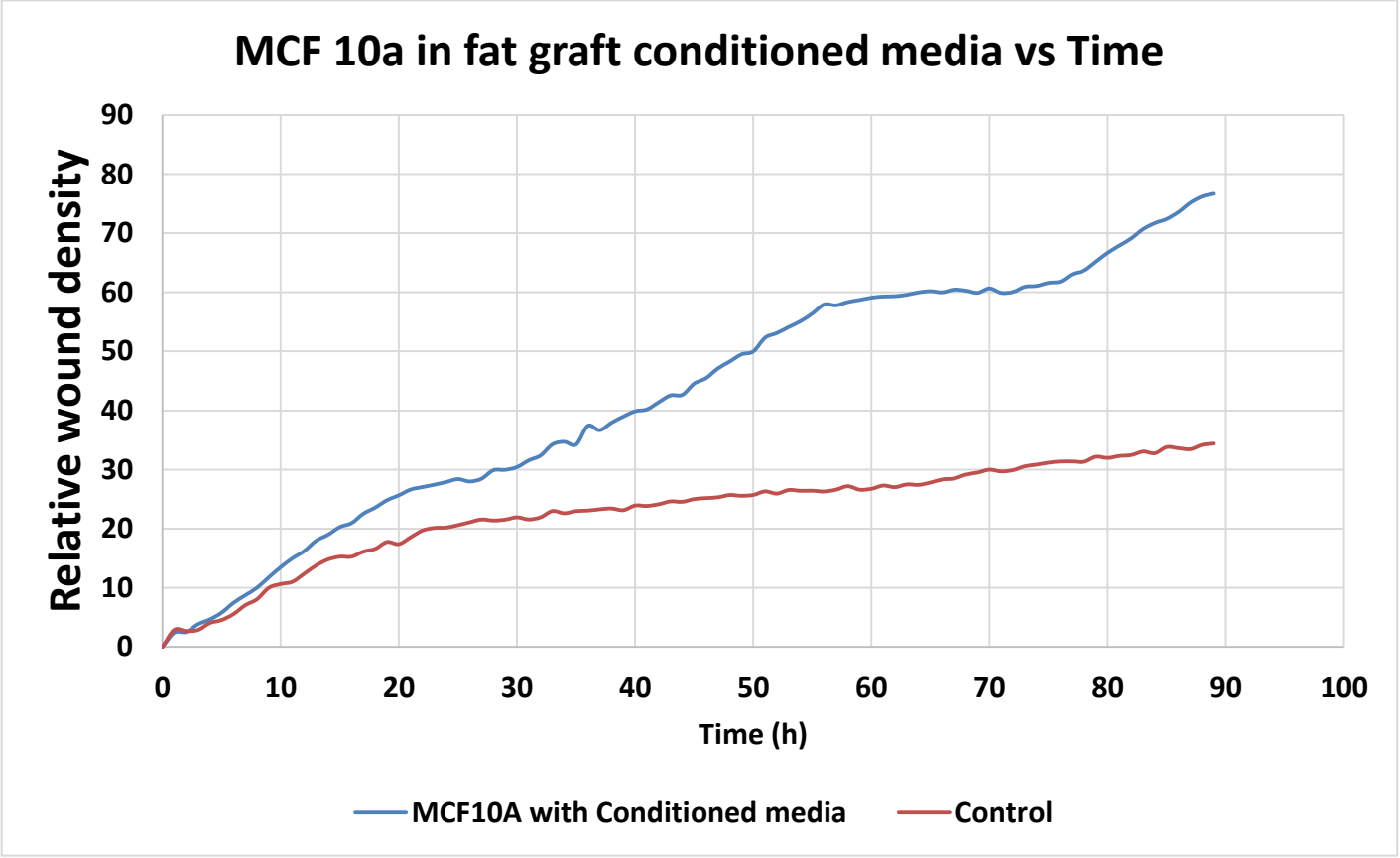


Figure 7-15 MCF10a Incucyte non-linear regression analysis

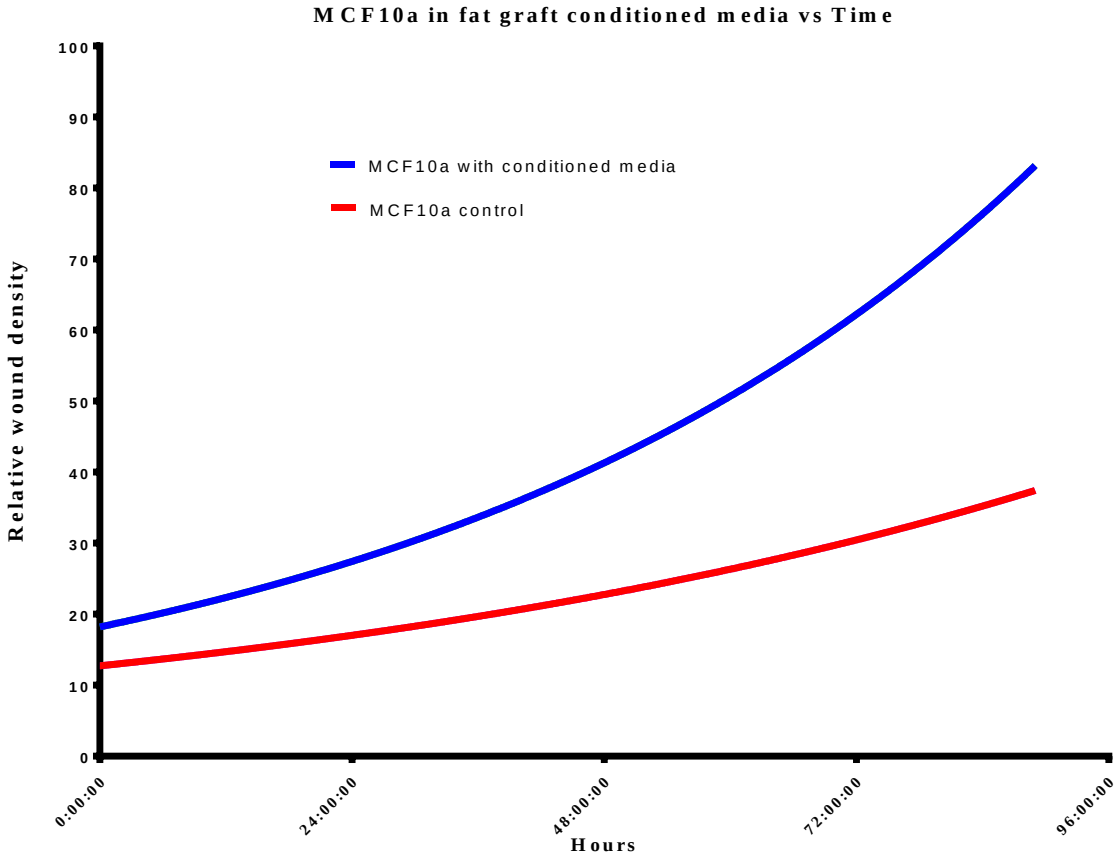


Table 7-8 MCF10a Incucyte non-linear regression analysis data

Curve being compared	Adjusted R square	p value	Confidence interval (Slope)
MCF10a Fat Graft Condition Media vs Control	0.8951 vs 0.8049	<0.0001	MCF10a Fat Graft Condition Media 0.1639 to 0.1923 Control 0.1126 to 0.1404

Figure 7-16 MCF10a Incucyte scratch assay time-lapse

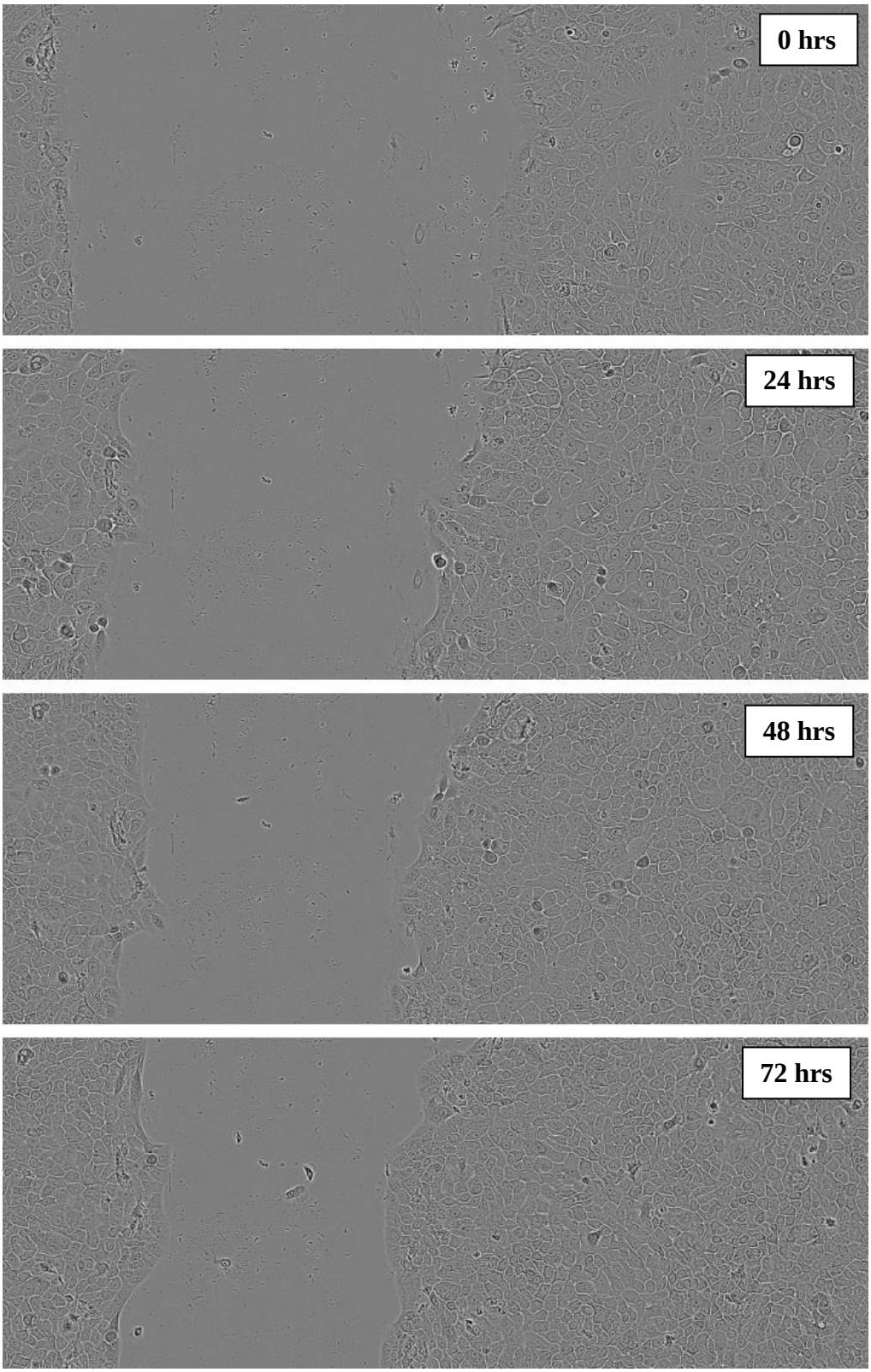


Figure 7-17 MCF7 growth curve

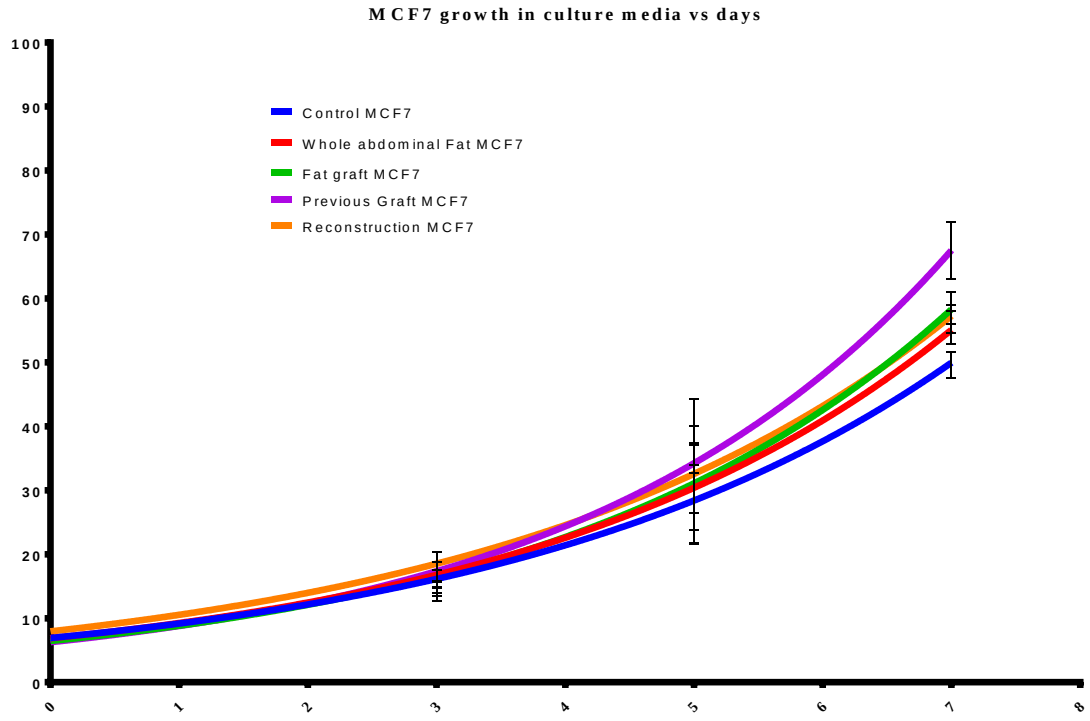


Table 7-8 MCF7 non-linear regression analysis

Curve being compared	Adjusted R square	P value	Confidence interval (Slope)
Control vs Whole abdominal Fat	0.9771 vs 0.941	0.1894	Control 0.1059 to 0.1393 Whole abdominal Fat 0.09 to 0.159
Control vs Fat Graft	0.9771 vs 0.9378	0.1038	Control 0.1059 to 0.1393 Fat Graft 0.1084 to 0.1773
Control vs Previous fat graft	0.9771 vs 0.9725	<0.0001	Control 0.1059 to 0.1393 Previous fat graft 0.1224 to 0.1687
Control vs Reconstruction	0.9771 vs 0.9564	0.0110	Control 0.1059 to 0.1393 Reconstruction 0.1076 to 0.16
Fat graft vs Previous fat graft	0.9378 vs 0.9725	0.0416	Fat Graft 0.1084 to 0.1773 Previous fat graft 0.1224 to 0.1687

7.4.3 MCF7 Non-linear regression analysis outcome

The results of the two-dimensional growth curves of MCF7 show increased proliferation of cells when placed with conditioned media from fat from different sources and this was significant for cells from previous fat graft and fat taken from a previous breast reconstruction.

7.4.4 MCF10a Operetta High Content Imaging System analysis of two-dimensional constructs cellular morphology differences

The Operetta High Content Imaging System was used for analysis of MCF10a and conditioned media two-dimensional constructs and the morphological changes related to nuclei roundedness and surface area (Figures 19 & 20). The MCF10a cells in standard media tended to have a higher nuclear size and surface area. The MCF10a specimens in conditioned media had smaller nuclear size and surface area.

Figure 7-18 Two-dimensional construct MCF10a with conditioned media: nuclei roundedness (mean per well)

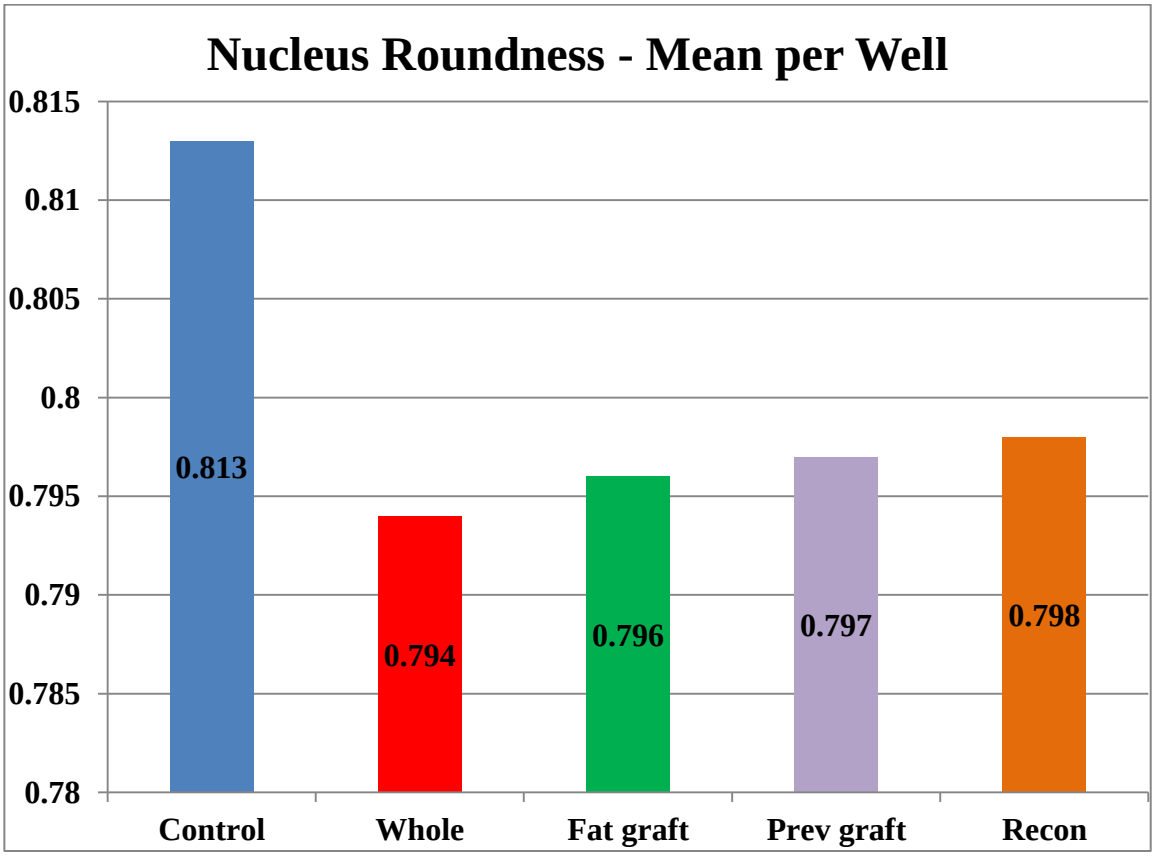
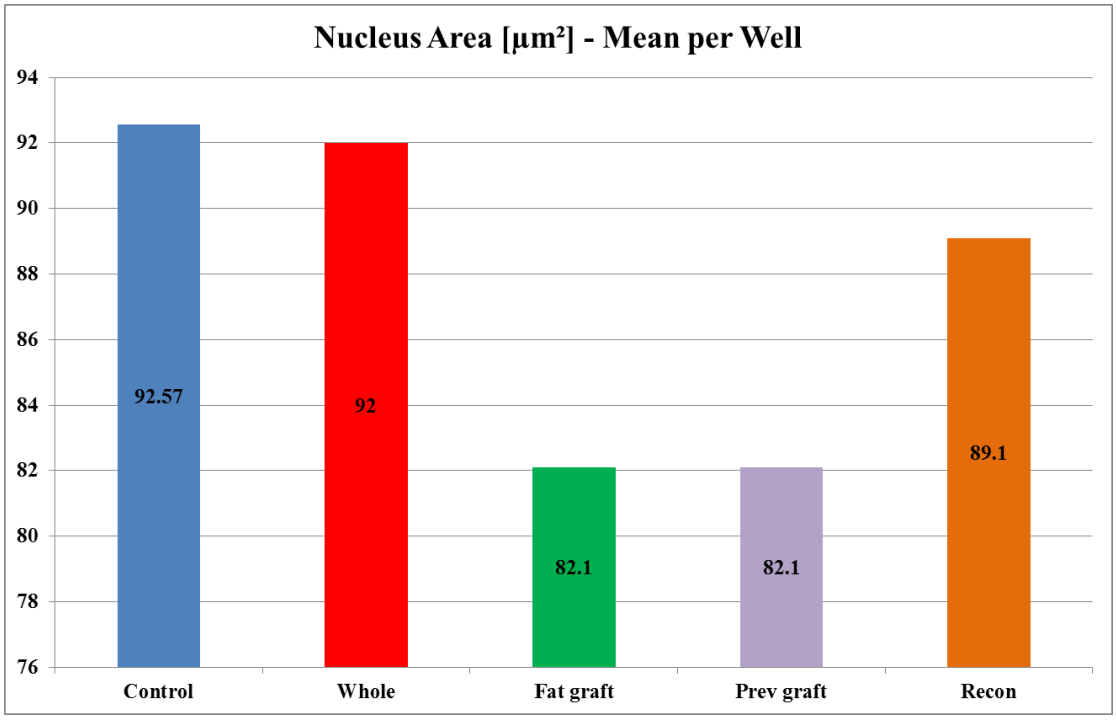


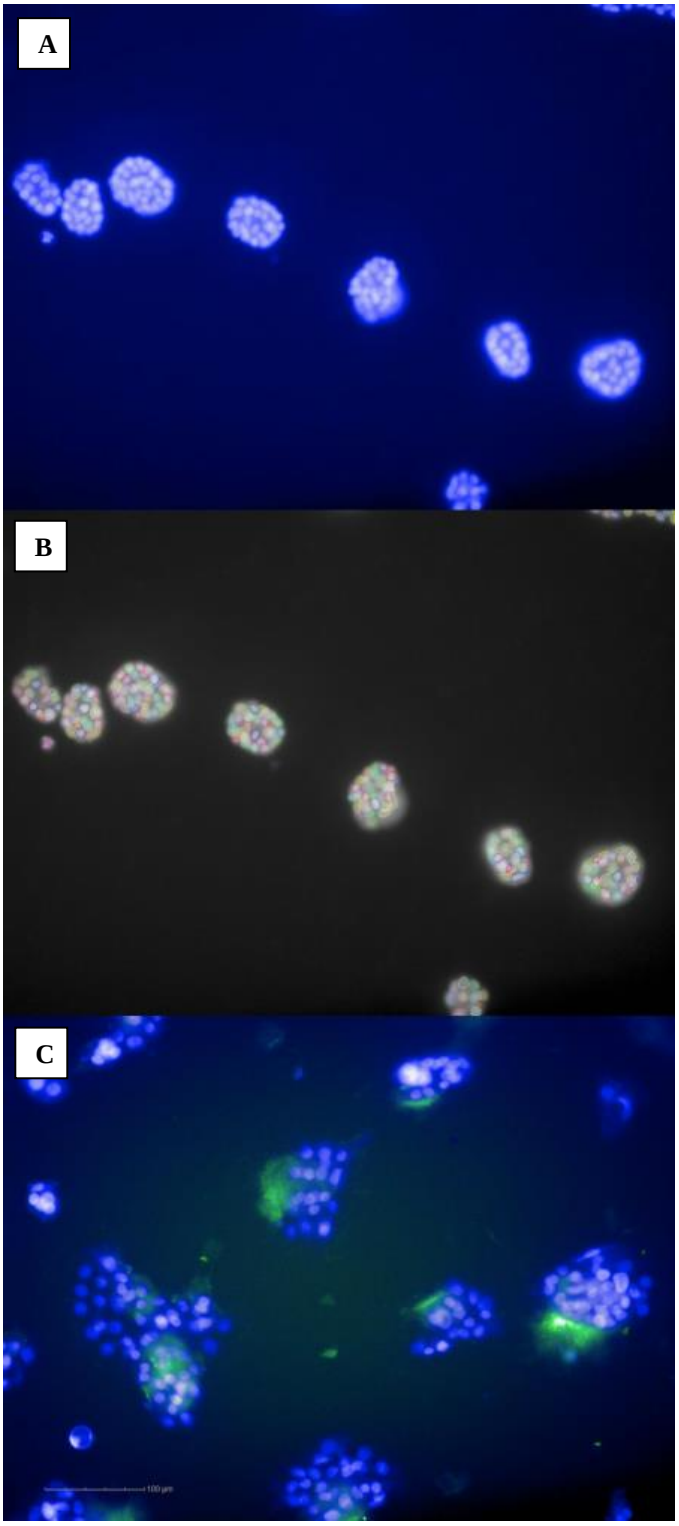
Figure 7-19 Two-dimensional construct MCF10a with conditioned media: nucleus area (µm^2) (mean per well)



7.4.5 Assessment of three-dimensional MCF10a cellular morphology differences using Operetta High Content Imaging System

The Operetta High Content Imaging System was used for analysis of three-dimensional MCF10a cells in standard media compare with MCF10a cells in conditioned media observing three-dimensional the appearance of three-dimensional mammospheres was captured using the Operetta's image capture (DAPI stain). The MCF10a cells in standard media kept the mammosphere shape (Fig 21 A.) whereas the three-dimensional MCF10a constructs in conditioned media seem to lose this mammosphere appearance (Fig 21 C.).

Figure 7-20 Operetta High Content Imaging System analysis of three-dimensional constructs: A) mammospheres in standard media (DAPI stain), B) measured nuclei dimensions, C) MCF10a cells in conditioned media.



7.4.6 MCF10a Operetta High Content Imaging System analysis of three-dimensional constructs cellular morphology differences

The Operetta High Content Imaging System was used for analysis of three-dimensional MCF10a cells in standard media compare with MCF10a cells in conditioned media three-dimensional observing morphological changes related to nuclei roundedness and surface area (Figure 22 & 23). The two-dimensional finding regarding nuclear size and surface area were validated by the three-dimensional results. MCF10a tended to be larger than the groups with conditioned media.

Figure 7-21 Analysis of nucleus roundness of three-dimensional MCF10a in standard media vs MCF10a cells in conditioned media

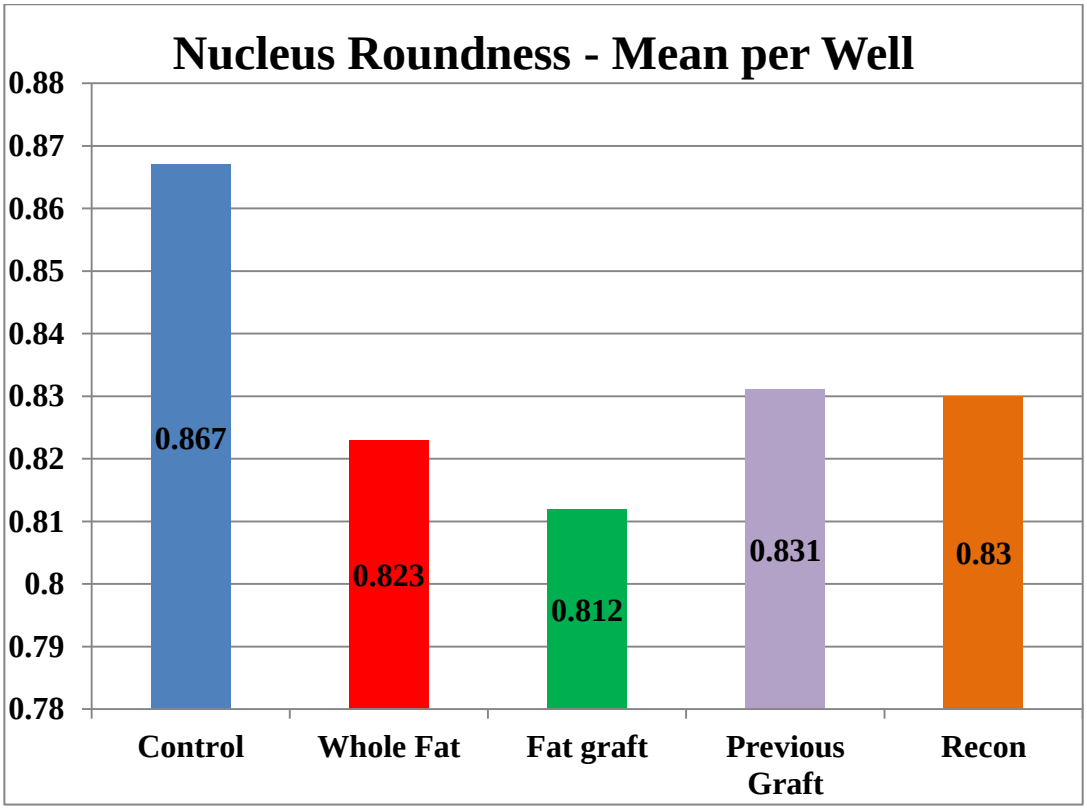
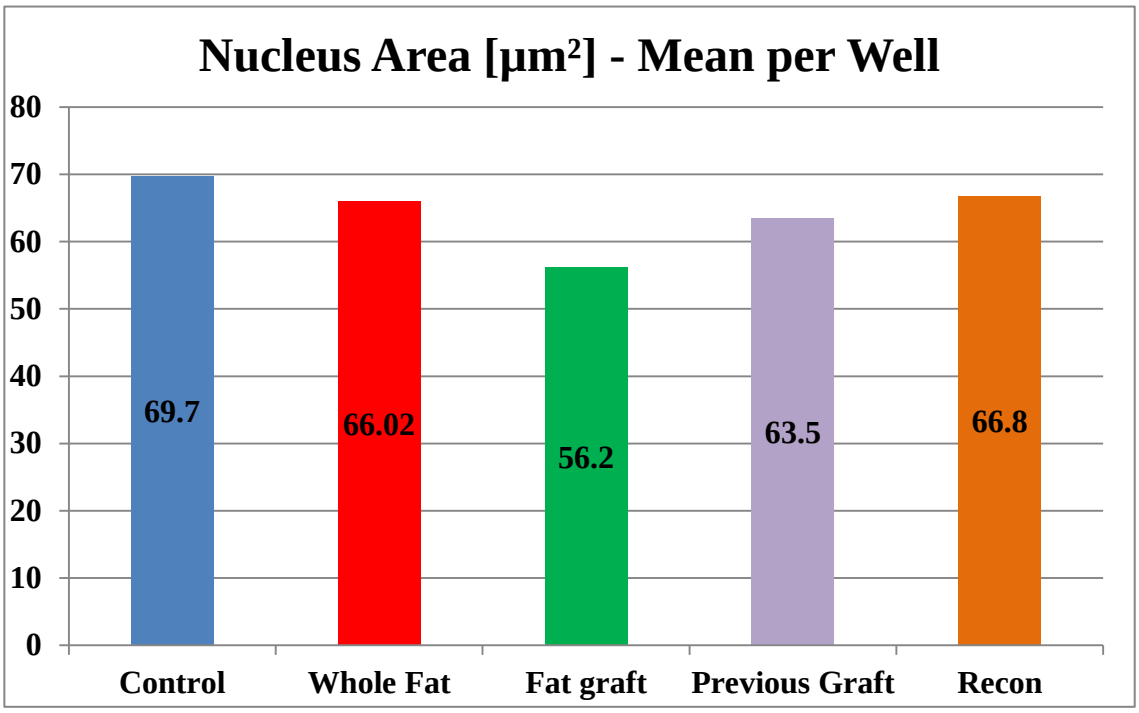


Figure 7-22 Analysis of nucleus area of three-dimensional MCF10a in standard media vs MCF10a cells in conditioned media three-dimensional



7.4.7 Two-dimensional photography of three-dimensional MCF10a morphological appearance

The confocal microscope was used to observe MCF10a and conditioned media three-dimensional constructs and the morphological changes in the cell appearance. The MCF10a cells in standard media tended to have more spherical cells (Figure 24 A.) whereas the MCF10a cells with adipocytes appeared to have cells which were in varying stages of morphological development (Figure 24 B.).

The two-dimensional imaging of the “adipocytes” revealed cells which again appeared more like preadipocytes than fully differentiated adipocytes (Figure 25 A & B). There did appear to be a relationship between the MCF10a cell and the preadipocyes as these were always adherent to each other in clusters suggesting possible to cell to cell cross-talk. The same was true for MCF10a cells in Cultrex® with standard MCF10a media (Figure 26 A.) and MCF10a with “adipocytes” from abdominoplasty specimens (Figure 26 B.) and fat from the breast reconstruction specimen (Figure 26 C.).

Figure 7-23 MCF10a in three-dimensional with the A) standard culture media and B) MCF10a in conditioned media.

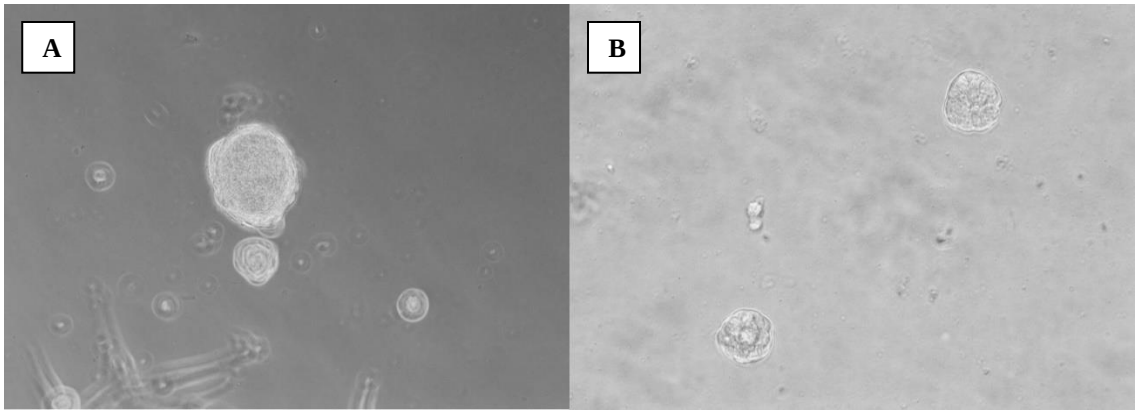


Figure 7-24 Individual three-dimensional MCF10a cell with "adipocytes" A) focus on MCF10a cell B) focus on the adipocyte

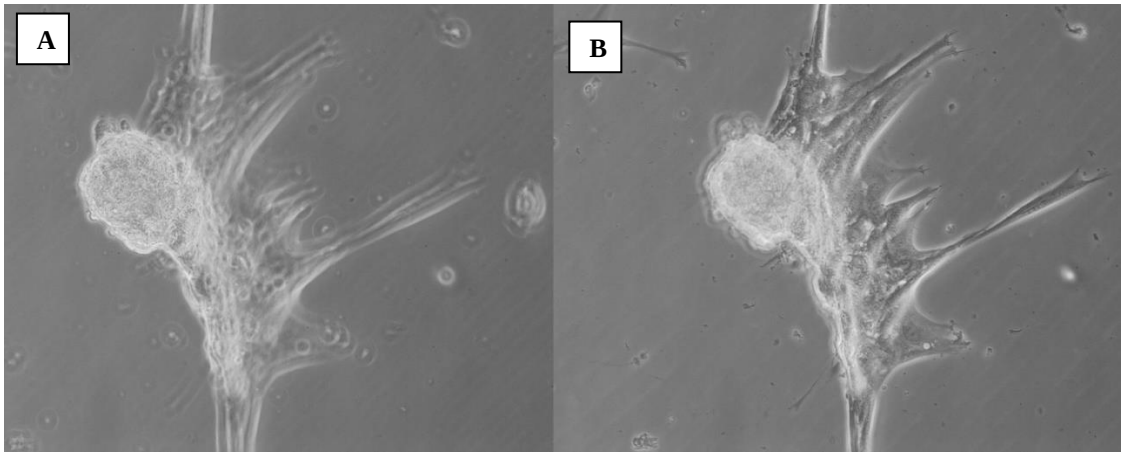
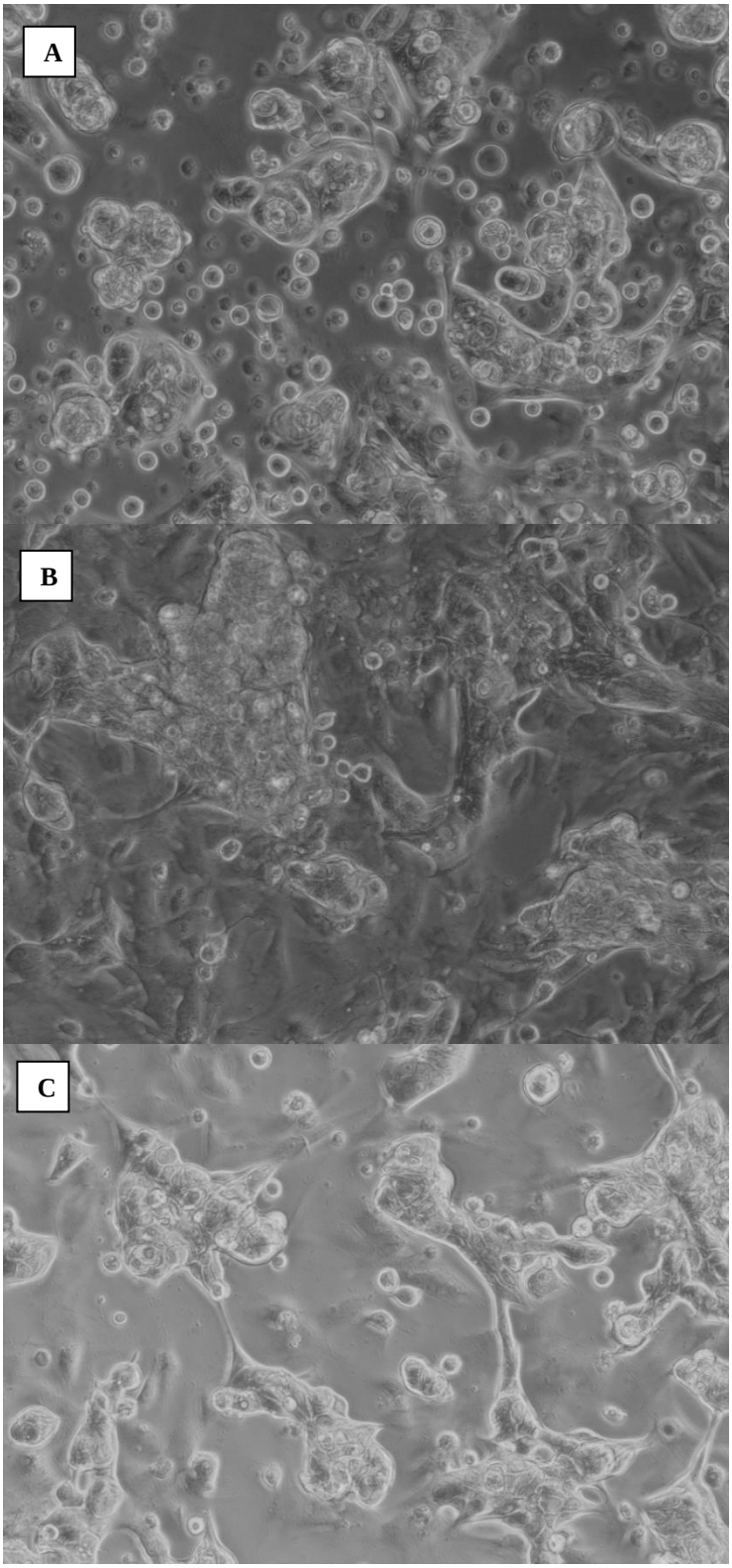


Figure 7-25 Three-dimensional MCF10a A) controls in media, B) whole fat adipocytes, C) reconstruction adipocytes.



7.5 Discussion

Most fat harvesting techniques involve the infiltration of a tumescent local anaesthetic mixture and subsequent aspiration of fat using perforated metal cannulae and suction pressure generating devices. Key differences between modern lipoaspiration techniques involve the use of centrifugation and additives to the graft itself. The Coleman technique, which was initially designed for correction of defects in the face was then adopted for preparation and injection of fat into the breast. This involved the harvest of fat from the abdomen, thighs or medial knees. The fat was then rinsed and centrifuged, in order to concentrate the fat, prior to injection into the breast. Rohrich et al. explored the efficacy of centrifugation of fat prior to injection and concluded that centrifugation did not improve the viability of grafted fat and was therefore not deemed to be absolutely necessary (Rohrich et al., 2004). The cell enrichment technique involves harvesting the fat and separating it into two layers; one layer of mature adipocytes and one layer digested in collagenase to concentrate the pre-adipocytes (Toyserkani et al., 2016). The pre-adipocyte layer is then re-introduced to the adipocytes, with the rationale that the more concentrated cellularity of the injectate should enhance the "take" of the graft (Bulstrode et al., 2001).

Other processing methods include washing and filtration, gravity separation similar to that used in Chapter 5, and gauze rolling (Smith et al., 2006, Fisher et al., 2013). Interestingly of these techniques gauze rolling may provide the greatest graft volume by minimising adipocyte loss, however it may have increased loss of stromal vascular fraction which may have preadipocytes and growth factors that promote adipocyte differentiation. The best method for fat graft harvest and preparation in order to yield maximum fat graft retention rates is an area of further investigation. Concentrating adipocytes appears to be one technique which hopes to improve graft viability in-turn; this may increase the concentration of adipose derived stem cells.

7.5.1 Behaviour of breast cells in the presence of autologous fat

The body consists of white and brown fats that serve different functions (Lidell and Enerback, 2010, Cannon and Nedergaard, 2004). These fat cells are held in an extracellular matrix (ECM) structure that imparts structural integrity against mechanical deformation (Mariman and Wang, 2010). Within this ECM is the stromal vascular fraction (SVF), which consists of a rich milieu of cells including: fibroblasts, macrophages, lymph, endothelial cells, multi-potent stem cells, and pre-adipocytes that can constitute up to 50% of the cells in the stromal vascular fraction. White adipose tissue stores energy and is composed of spherical adipocytes with a lipid droplet that fills the volume of the cell with a diameter of 30-130 μm (Timmons et al., 2007). Brown fat is a thermoregulatory assisting in changing body temperature during cold temperatures (Cannon and Nedergaard, 2004).

Pre-adipocytes begin differentiation during embryonic stages of development. The cell line that commonly differentiates into adipocytes is the 3T3L-1 cell line and it does this via cell to cell contact until confluence is reached (Krawisz and Scott, 1982). The current study incorporated various regulators that promote differentiation into adipocytes in the differentiation media including: insulin, foetal bovine serum, dexamethasone, and biotin. During differentiation, the changes that occur in the cell morphology of the pre-adipocytes include a shift from an elongated fibroblast shape to a more spherical shape with observable lipid accumulation. This is the hallmark of adipogenesis. At this point, it is possible to stain the adipocytes with Oil red O stain (Lee and Fried, 2014).

Pre-adipocyte lipogenesis is complex. Cross-talk between pre-adipocytes induces type VI collagen and LPL release, which in turn activates c-myc, junB, c-jun, and c-fos. Those four proto-oncogenes regulate the CAAT/enhancer-binding proteins' (C/EBPβ and C/EBPδ) subsequent activation of proliferator-activated receptor

gamma (PPARϒ), which promotes insulin sensitivity, lipogenesis, and lipolysis (Huang et al., 2009). Adipocyte formation is also governed by the following regulators: ribosomal protein S6 kinase 1 (S6K1), WNT inhibition, hedgehog (HH) signalling pathway, glutathione, transforming growth factor β, insulin like growth factor 1 (IGF1), bone morphogenetic proteins (BMPs), and activin (Huang et al., 2009, Zamani and Brown, 2011, Kawai and Rosen, 2012, Zuniga et al., 2010, Widberg et al., 2009, Cousin et al., 2007). These function by either inducing the hypertrophy or hyperplasia of adipocytes.

In normal, low inflammation states, adipocyte expansion occurs with minimal involvement of the extracellular matrix. Pathologically, growth can be arrested by pro-inflammatory processes. Adipokine secretion promotes a pro-inflammatory state, as does leptin, resistin, and visfatin. There are advantages to the mounting of an inflammatory response, as adiponectin has been reported to inhibit tumour necrosis factor (TNF) and interleukin 6 (IL-6) as well as IL-10 and IL-1RA, thereby promoting an anti-inflammatory response.

When adipocytes are transferred as a graft (i.e. without an associated blood supply) the following has been observed to occur: firstly, degenerative changes related to apoptosis, macrophage-induced phagocytosis and necrosis, secondly, elevated expression of hypoxia-inducible factor 1α, which assists in homeostasis and vascularisation through angiogenesis (Ziello et al., 2007) and thirdly, elevated fibroblast growth factor 2 (Suga et al., 2010), which was implicated by Liu et al. to result in transient MAPK activation and subsequent MCF7 proliferation (Liu et al., 1998). This may be one explanation for the MCF7 growth rate curves in this study.

During the harvest of conditioned media, the volume of pre-adipocytes in each harvest was higher than predicted. Non-quantitative assessment of the thickness of

the pre-adipocytes vs adipocyte layer found layers to be of comparable thickness macroscopically. Given pre-adipocytes are much smaller in dimension, which suggests there may be much higher numbers in any sample of fat graft compared with the number of adipocytes. In culture, pre-adipocytes proliferate quickly and are easy to culture. Suga et al. investigated tissue remodelling under ischaemic conditions and concluded that although adipocyte death occurs, there is still activation of progenitor cells within this environment (Suga et al., 2010). Whether pre-adipocytes can be frozen and then thawed for use in patients during repeat procedures could be an area of exploration. Conversely, the verdict as to the deleterious effects versus benefits of adipose-derived stem cells remains in contention.

In order to explore the relationships between fat tissue and breast cells *in vitro*, two-dimensional cell culture experiments are now being used in conjunction with *in vitro* three-dimensional models.

7.5.1.1 Behaviour of benign breast cells in a microenvironment replicating autologous fat transfer – evidence from two-dimensional culture

There are several phases which typically occur for the growth of cells in either two-dimensional or three-dimensional culture. An acclimatisation phase in which cells are taking in nutrients and begin to multiply. This occurs in continuity with the lag phase (days 0-5) that includes cell growth and division. The log phase is a period in which cells will undergo exponential growth depending on the level of nutrient availability. This is then followed by a stationary phase: once provisions are exhausted and the cells are confluent, they remain in a stationary phase in which cell growth is arrested and a phase of cell death: cells begin to die, either from the toxins

that are released from the cells or from limited nutrients.

Comparative growth of non-malignant (MCF10a) breast cells in media conditioned with different types of fat samples in a two-dimensional model growth curve demonstrated that the cells of each group reached the log phase between days 3 and 5. In the log phase growth curves were comparable between each of the culture environment groups. The whole abdominal fat, fat graft, and fat from a previous reconstruction well all surpassed the growth of the control cells between days 5 and 7. The exception was the group with media conditioned with fat from a previous autologous fat graft site, which showed minimal growth in the log phase.

There was no significant difference when comparing the growth curves between the control group and the whole abdominal fat, fat graft, and previous reconstruction fat groups. The difference in the rate of growth between the fat graft (lipoaspirate) and the previous graft specimens was significant. The implication of this may be that the adipose-derived stem cells injected initially enhance the growth of normal breast cells. In the long term, however, once the fat has taken (vascularised) or the pre-adipocytes have been activated, there is no long-term enhancement of the growth of normal breast cells.

Interestingly, when placed in the Incucyte scratch assay, the MCF10a cells that were cultured in fat graft media grew significantly faster than the controls. This may be attributable to the apocrine effect of the fat graft conditioned media on MCF10a growth, which is in keeping with the growth curve results. Alternatively, the control cells may have exhausted the nutrients from their media. Once cells are placed into the Incucyte, they could not be removed in order to replenish the culture media. However, these results were an average of a technical triplicate, which gives it a degree of strength. A limitation of this experiment was the lack of biological

replicates as the experiment was only run once. For further validation of the results, it would be necessary to run this investigation at least two more times.

Normal mammary cells have previously been demonstrated to undergo transformation when placed in the bed of mouse fat pads that have been irradiated (Barcellos-Hoff and Ravani, 2000). In certain microenvironments, they interact with surrounding stroma, which can alter normal breast cells by triggering them to undergo malignant transformation. This may be either a form of biomechanical or biochemical change. Yusuf et al. investigated the biochemical changes that MCF10a cells undergo once they receive growth cues. Changes in morphology were related to epidermal growth factor (EGF) and E2 binding to oestrogen receptors (Yusuf and Frenkel, 2010). The mechanism by which these regulators increased proliferation was by increasing mitogenic activity and the receptor-based activation of transcription errors (Fishman et al., 1995). These regulators are distinct from the previously described paracrine factors associated with adipocytes or pre-adipocytes and they may in fact derive from the tumour stroma (Huang et al., 2009, Zamani and Brown, 2011, Kawai and Rosen, 2012, Zuniga et al., 2010, Widberg et al., 2009, Cousin et al., 2007). Other factors associated with mammary carcinogenesis include chronic infection and inflammation leading to oxidative stress, which are biomechanical stressors that can alter cellular machinery (Yusuf and Frenkel, 2010). These are also unrelated to the process of fat graft injection. Inflammation may play a minor role, but it would be unlikely to be involved in the degree that may result in tumorigenesis. Adipocytes and pre-adipocytes are also unlikely to cause oxidative stress with resultant DNA damage.

7.5.2 Effects of different fat sources on the morphology of benign breast cells

Kiosses et.al. discuss MCF10a cell morphology and describe smaller sizes being associated with cells that are undergoing proliferation and replication(Kiosses et al., 2001). Once MCF10a cells have slowed development and are stationary they have a cobblestone appearance with large and round nuclei. The nuclear size and roundedness measured by the Operetta in the two-dimensional and three-dimensional models demonstrated that the nuclei were smaller and less round in the adipose tissue-conditioned media groups compared with those in the control group.

The two-dimensional nuclei were smallest in the fat graft and previous fat graft groups. The latissimus dorsi reconstruction, whole abdominal fat, and control groups showed comparably sized nuclei, and the whole fat was the smallest overall, falling below those of the fat graft and previous graft groups. When observing the nuclei roundedness, the whole fat group had the least round nuclei when compared to the roundedness of nuclei in the controls. This was a contrasting finding when observing cell size suggesting the whole fat group were large, but not round. However, the roundedness difference compared to the other groups ranged from a negligible difference of 0.001-0.004 μm.

In the three-dimensional model, with the introduction of Cultrex®, cell nuclear size validated the findings from the two-dimensional study, which showed that the fat graft and previous graft groups had the smallest nucleus, followed by the whole fat and latissimus dorsi reconstruction groups. The fat graft was found to have the least round nucleus, followed by the whole fat. The previous graft and previous reconstruction groups were similarly round, with the control group showing the roundest nuclei.

Micro-environment alterations can influence MCF10a growth and it seems that a fat graft and a history of previous grafts could hinder the ability of the MCF10a cells to remain stationary. Therefore, they may be replicating at a higher rate with more cells appearing with microfilament-rich filopodia and with smaller, less round nuclei (Kiosses et al., 2001). It may be that the cells with a larger polarized egg appearance are those that are more well-formed and have undergone contact inhibition. The cobblestone appearance of MCF10a as they sit in a confluent position may represent the larger cell. As Kiosses et al. discussed, normally 75% of MCF10a are stationary with the remaining cells being motile cells. In the presence of adipose tissue, it may be that the composition shifts toward having more motile cells and less of a cobblestone appearance. During their motile stage, they elongate under rapid changes to their microfilaments, become asymmetrical, and have fan-shaped lamellipodia (Kiosses et al., 2001).

7.5.3 Effects of different fat sources on benign breast cells in three-dimensional culture

Three-dimensional models have, up until recently, been used mainly in *in vivo* models. Mice studies are common to replicate the architecture of human tissues. In contrast to the cell monolayers produced in two-dimensional culture models 3D model provide comparable insights to sometimes more costly *in vivo* models. Matrigel® matrix basement membrane (Corning, 836 North Street, Building 300 Suite 3401 Tewksbury MA 01876, USA) is a well-established construct for three-dimensional cell culture. However, Matrigel, was not optimal for the experiments in this study because it is known to contain growth factors that may stimulate the growth of cells (Cronin et al., 2004). In the presence of tumour cells, tumour proteases break down matrigel to release growth factors (laminin-1 and collagen IV) enhancing proliferation through angiogenesis (Wang et al., 2011, Taqvi and Roy,

2006, Schneider et al., 2010) (Kleinman and Martin, 2005). Cultrex® has less of a growth promoting effect by decreasing its concentration to a standard of 15mg/mL and was therefore chosen for these studies (Cultrex® product data sheet produced by Trevigen.)(Benton et al., 2009, Benton et al., 2011).

The growth of the MCF10a cells in three-dimensional culture validated the two-dimensional findings. MCF10a cells in standard MCF10a media demonstrated spherical cells with a larger surface area than all other groups. The fat graft group which consisted of MCF10a cells in conditioned media from lipoaspirate demonstrated MCF10a cells which were small and less spherical compared to the control group, MCF10a cells cultured in conditioned media taken from the adipocytes from a women who had previously undergone autologous fat grafting for breast reconstruction and MCF10 cells cultured in condition media from abdominoplasty adipocytes. These findings may suggest altered effects on benign breast cells when placed with fat from different parts of the body.

7.5.4 Effects of different fat conditioned media on the proliferation of breast cancer cells- two-dimensional microenvironment

The role of adipose tissue in cancer development in experimental studies has shown that through endocrine and paracrine activity, adipose tissue resident progenitor cells produce growth factors that can act on nascent cancer cells. Lohsiriwat's experimental studies show that pre-adipocyte and progenitor cells can stimulate angiogenesis and cell growth (Lohsiriwat V, 2011). Another theory, by the same author, proposes the existence of a cell-signalling pathway of leptin on oestrogen-dependent and independent cancer cell types. Current pre-clinical studies on this subject are inconclusive. Qiao et. al. showed the inhibition of cancer proliferation via adipose-derived stem cell NF-KappaB downregulation and inhibition of Wnt signalling (Qiao et al., 2008). Another group showed breast cancer progression in a

murine mouse model with human mesenchymal stem cells obtained from lipoaspirate (Martin-Padura et al., 2012). One clinical study by Petit et al. involving fat grafting and lipoaspirate specifically outlined a need for more pre-clinical research into the paracrine effects of fat and breast cell lines (Petit JY, 2011).

Despite concerns, the benefits of adipose-derived stem cells are becoming well established. Di Summa et al. used adipose-derived stem cells (ADSCs) to regenerate the sciatic nerve in place of using other conduits for nerve reconstruction (di Summa et al., 2010). In a prospective clinical study, Bruno et al. identified the decreased expression of P53 in scars after fat grafting, which indicated increased cellular proliferation in the scar due to the fat grafting (Bruno et al., 2013). However, the increased expression of Ki-67 and P53 may also potentiate the growth of tumours. This aligns with previous evidence that while ADSCs can stimulate active cancer cells, they do not have any effect on dormant cells. Therefore, if cancer cells are active and untreated, a proliferation of cells will occur (Smith et al., 2002). The results of the two-dimensional growth curves of MCF7 show increased proliferation of cells when placed with conditioned media from fat from different sources, supporting previous theories. Unfortunately, this data could not be validated by the MCF7 three-dimensional experiment and in order to determine this finding conclusively would need to be shown in both three-dimensional and *in vivo* models.

Currently the literature on fat grafting and breast cancer cells outlines explicit warnings regarding the use of adipose-derived stem cells, as they may assist in the proliferation of quiescent breast cancer cells (Krause et al., 2008, Lohsiriwat V, 2011, Petit et al., 2012). Chandler et al. explained that although tumour cells inhibit adipocyte differentiation, they might promote pro-angiogenic factor secretion and myofibroblastic differentiation (Chandler et al., 2012). They concluded that adipose-derived stem cells placed in the microenvironment with tumour cells do

potentiate growth.

The findings from the MCF7 growth curve are in keeping with concerns raised in the previous literature, with a significant increase in the numbers of MCF7 cells for the groups treated with conditioned media from the fat graft, previous graft, and previous reconstruction specimens. However, the number of MCF7 cells in the conditioned media taken from the abdominoplasty specimens was not significantly higher than that of the control group.

In contrast to the MCF10a findings in this study, the previous graft had the highest growth rate of MCF7 cells. This was significantly higher than the fat graft MCF7 growth rate. This may suggest that once in situ, a previous graft may display more oncogenic characteristics than the other types of adipose tissue. At least, fat graft has been implicated in previous articles warning against the use of fat graft in women who have had breast conserving surgery, such as the invasive DCIS retrospective cohort study by Petit et al. which was supported in the findings from the MCF7 cell growth curve. However, Chandler et al. suggests that it's the reverse relationship. They investigated the tumour-secreted soluble factors that affect the capabilities of adipose-derived stem cells to differentiate and display pro-angiogenic capabilities (Chandler et al., 2012). Tumour cell-derived TGF-β, provides physicochemical cues that induce adipose-derived stem cell phenotypic changes, resulting in their transformation into myofibroblasts, which make up the pro-angiogenic cellular component of the tumour stroma (Chandler et al., 2012).

Regardless, the two-dimensional results of the current study would need to be validated further in three-dimensional models, including a mouse model. Presumably, if breast cancer cells are placed with adipose-derived stems cells in an *in vivo* model, they will grow more rapidly than with an MCF7 cell line alone. A In

practice, a woman would need to have invasive disease at the time of autologous fat grafting or during the time period in which the fat graft is remodelling in the new breast microenvironment in order to potentiate the growth of cells. Patient selection considerations include those that are deemed low risk for breast cancer recurrence as outlined in Chapter 5.

7.5.5 The interaction between mature adipocytes and breast cells

The pre-adipocytes that were harvested during this study were abundant in all specimens. Given the hypoxic environment into which the fat graft is placed once separated from its vascular supply, it may be that this robust cell, which is readily proliferated in vitro in high numbers, is activated by oxidative stress to differentiate into pre-adipocytes.

Despite the observation of lipid accumulation during pre-adipocyte differentiation, the Oil red-O stain failed to stain the adipocytes. A few explanations of this include the permeabilising capability of Triton-X 100, which can 'punch holes' in the walls of cells and may have assisted the entry of the Oil red-O stain into the cells. This was explored subsequently, but it too failed to stain the cells. Another protocol that included thyroxine in the recipe was found, but time was the rate-limiting step to performing further experimentation using thyroxine in the differentiation media. In order to further confirm the formation of adipocytes, the expression of adipogenic markers such as the adipogenic transcription factors PPAR γ, CEBP β, and CEBP α, may also be included in confirmatory tests. Otherwise, it may be possible to test for adipocyte genes like lipoprotein lipase, binding protein 4, perlipin, leptin, and adiponectin. This should be considered in any future experiments.

Although whether or not the pre-adipocytes had differentiated was not determined, there did seem to be a propensity for the fat cells to migrate to MCF10a and MCF7 cells (shown in previous images). Therefore, cross-talk between the cells may be inhibitory, but it is more likely to enable a symbiotic relationship that benefits the MCF cells in some respect. Indeed, this was demonstrated by Chandler et al., whose transwell migration assay demonstrated that Tumour Conditioned Media (TCM) attracted adipose-derived stem cells towards their location (Chandler et al., 2012). Potter et al. showed a similar finding that stromal chemokines like CCL2 increased breast cancer epithelial cell migration, therein harnessing stromal cell biology by increasing tumour size (Potter et al., 2012).

7.5.6 Study limitations

One limitation of this study was the small number of women who were sampled for specimens. It may have been ideal to take several samples from the participants in the previous reconstruction group. Further studies could also include other cell lineages such as MDA-MB231-TCM breast cancer cells and isogenically-matched fully malignant MFC10-CA1a cells, as well as pre-malignant MCF1aAT cells. Outside of the use of the Operetta, a three-dimensional *in vivo* mouse model with MCF10a and adipocytes in Cultrex® could be an area of investigation to validate the two-dimensional findings. The use of MCF10a cells may be suitable in nude mice because they are non-tumourigenic (Soule et al., 1990).

7.6 Conclusion

Further research is required to validate the findings from the two-dimensional model which demonstrated the increased cell proliferation of MCF10a and MCF7 cells when placed within adipose-derived conditioned media obtained from fat graft, previous fat graft, latissimus dorsi, and abdominoplasty adipose tissue specimens. In regards to the question of whether fat grafting creates a more dangerous microenvironment for the growth of residual cancer cells when compared to the normally present sub-cutaneous fat, it would appear that the engrafted fat does induce higher rates of proliferation in breast cells and therefore could be a cause for concern.

Breast reconstruction tissue also appeared to potentiate the growth of a breast cancer cell line when compared with subcutaneous fat, but to a lesser extent than both the grafted fat and previous fat graft specimens. In the case of a normal breast cell line, the fat graft promoted a greater rate of proliferation, though this may be subject to change over time once the fat graft is integrated into the new breast microenvironment. Although these findings align with previous concerns raised in the literature regarding the use of adipose stem cells in women who have previously undergone mastectomy for breast cancer is a fertile area for further research investigating its safety and efficacy.

Chapter 8. Thesis Conclusions

Autologous fat grafting is being used increasingly in a clinical setting for breast reconstruction. In order to establish deficits in the current literature, all clinical studies were systematically reviewed identifying articles including women who have undergone breast conserving surgery or total mastectomy and then fat grafting. There remains a paucity of high quality clinical research on the outcomes of autologous fat grafting. This was shown using NHMRC guidelines and STROBE assessment checklists. The safety and efficacy of autologous fat grafting for defect correction post breast conserving surgery and for whole breast reconstruction needs to be explored with prospective clinical studies.

First, it was necessary to establish whether or not women who have undergone breast cancer surgery would consider surgical remediation using autologous fat grafting and what their quality of life is compared with a population of women who have not had breast cancer surgery and women who have undergone total mastectomy and breast reconstruction. The BREAST-Q and an adjunct questionnaire were used to establish quality of life outcomes in women who had undergone breast cancer surgery. These results were compared to a group of women who had not had breast cancer surgery and women who have undergone total mastectomy and breast reconstruction. Of the women who had undergone breast conserving surgery 15% would consider surgical remediation with autologous fat grafting as an option. Women who have undergone total mastectomy and breast reconstruction for cancer achieve a good quality of life, and the quality of life outcome was at least as good as that achieved following breast-conserving surgery. Furthermore, breast conservation was associated with more pain and discomfort in the chest area and poorer sexual well-being outcomes than mastectomy and

reconstruction. This information suggests that the quality-of-life outcomes in women undergoing total mastectomy and breast reconstruction might actually exceed the expectations of most patients with breast cancer. The use of autologous fat grafting in women who have undergone breast conserving surgery and total mastectomy is an area that warrants further investigation.

A proof of concept clinical case in which a woman underwent whole breast reconstruction with autologous fat grafting in the context of previous rotation flap approach mastectomy demonstrated promising results. This confirmed the potential for fat grafting as an option for whole breast reconstruction. However, whether or not there are ways of improving efficacy of the fat grafting procedure required further exploration using validated outcomes measures, specifically, determining fat graft retention rates over time.

A prospective study was then designed to investigate patient satisfaction and the efficacy of the BRAVA device and autologous fat grafting for breast reconstruction in women who had undergone breast cancer surgery. This was measured using the validated BREAST-Q patient reported outcome measure and demonstrated improved women's quality of life. Notably, women who had undergone radiotherapy experienced softening of previously radiotherapy damaged tissue after autologous fat grafting. However, in terms of efficacy, using a 3D laser scanner and breast MRI, the amount of fat graft retention over time was less than expected. This resulted in a need for multiple autologous fat grafting procedures to achieve a woman's desired result. During the study period, the three-dimensional laser scanner was shown to be equivalent to non-contrast MRI for the assessment of breast volume, further validating the 3D laser scanning technology. Given the likely lower cost and convenience of laser scanning compared to MRI, this is relevant for

plastic surgeons performing complex breast reconstruction work. Although there were no instances of locoregional recurrence in the prospective study over the course of 12 months the question remained as to whether or not adipose derived stem cells, within fat graft, alter proliferation and morphology of normal breast cells and breast cancer cell lines.

In order to address this area of contention, the *in vitro* study sought to address concerns regarding breast cell proliferation and changes in breast cell morphology. When a benign breast cell line (MCF10A) and malignant breast cell line (MCF7) cells are placed in media from adipose-derived conditioned media (obtained from fat graft, previous fat graft, latissimus dorsi breast reconstruction, and abdominoplasty adipose tissue specimens) they show increased proliferation rates and altered morphology compared to control cell lines in two-dimensional culture. For benign breast cells these finding were validated in three-dimensional culture. These findings warrant further investigation with *in vivo* studies. Interim caution should be maintained regarding possible deleterious effects of injecting adipose derived stem cells into the breast post breast cancer surgery until relational research has been conducted.

In vitro and the clinical studies contained in this thesis have shown that there is a role for autologous fat grafting for breast reconstruction in women who have undergone breast cancer surgery. However, further high-quality, ethically approved, prospective clinical research trials need to be conducted before fat grafting is used without clinic equipoise regarding locoregional recurrence. Eventually, the benefit of adipose derived stem cells and their role in tissue regeneration may be discovered and used in the realm of breast reconstruction to improve outcomes for women who have undergone breast cancer surgery.

Appendix 1. NHMRC Evidence Hierarchy

Level	Intervention [1]	Diagnostic accuracy [2]	Prognosis	Aetiology [3]	Screening Intervention
I [4]	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A study of test accuracy with: an independent, blinded comparison with a valid reference standard,[5] among consecutive persons with a defined clinical presentation[6]	A prospective cohort study[7]	A prospective cohort study	A randomised controlled trial
III-1	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)	A study of test accuracy with: an independent, blinded comparison with a valid reference standard,[5] among non-consecutive persons with a defined clinical presentation[6]	All or none[8]	All or none[8]	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)
III-2	A comparative study with concurrent controls: ▪ Non-randomised, experimental trial[9] ▪ Cohort study ▪ Case-control study ▪ Interrupted time series with a control group	A comparison with reference standard that does not meet the criteria required for Level II and III-1 evidence	Analysis of prognostic factors amongst persons in a single arm of a randomised controlled trial	A retrospective cohort study	A comparative study with concurrent controls: ▪ Non-randomised, experimental trial ▪ Cohort study ▪ Case-control study
III-3	A comparative study without concurrent controls: ▪ Historical control study ▪ Two or more single arm study[10] ▪ Interrupted time series without a parallel control group	Diagnostic case-control study[6]	A retrospective cohort study	A case-control study	A comparative study without concurrent controls: ▪ Historical control study ▪ Two or more single arm study
IV	Case series with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard)[11]	Case series, or cohort study of persons at different stages of disease	A cross-sectional study or case series	Case series

Appendix 2. STROBE Statement Checklist

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Appendix 3. Breast area record for lipografting: Version 1

Trial #284

Government of South Australia
SA Health

FLINDERS MEDICAL CENTRE

Plastics and Reconstructive Surgery Department

BREAST AREA RECORD FOR LIPOGRAFTING

Date: ...../...../.....

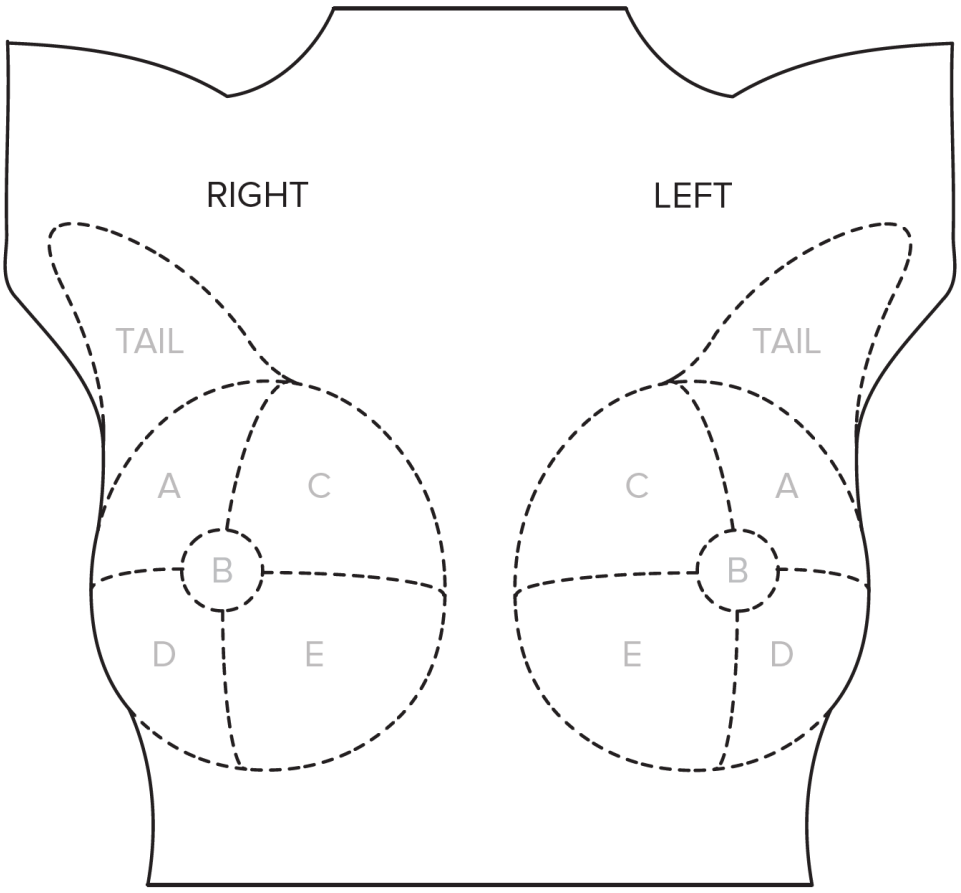
Affix patient label here

Right Breast

- ☐ Previous mastectomy and reconstruction
- ☐ No reconstruction
- ☐ Previous Partial
- ☐ Other .....

Left Breast

- ☐ Previous mastectomy and reconstruction
- ☐ No reconstruction
- ☐ Previous Partial
- ☐ Other .....



Sub-cutaneous – RED
Intra-glandular – GREEN
Retro-glandular – BLUE
Intra-muscular – YELLOW

A= upper outer quadrant
B= Nipple and areolar
C= upper inner quadrant
D= Lower outer quadrant
E= Lower inner quadrant

Sign:..... Designation:..... Print:.....

Appendix 4. Breast area record for lipografting right side: Version 2

Government of South Australia
SA Health

FLINDERS MEDICAL CENTRE

Affix patient label here

Plastics and Reconstructive Surgery Department

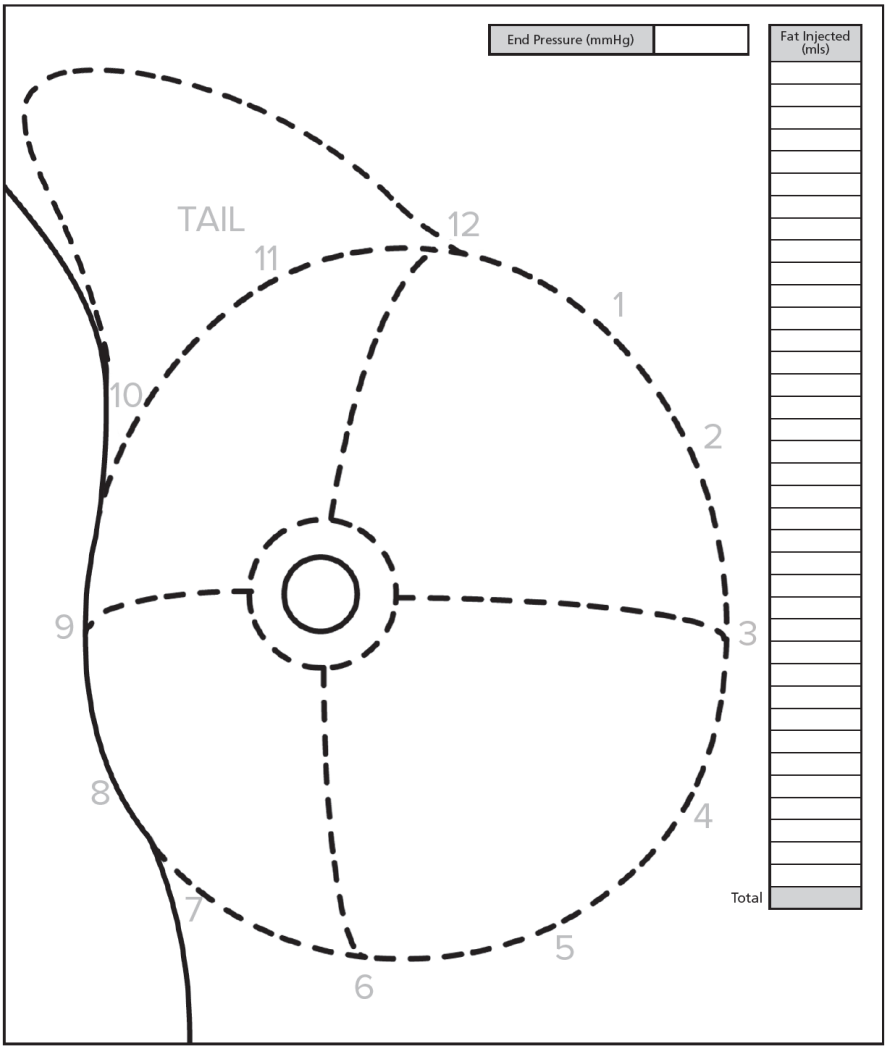
Breast Area Record For Lipografting

Right Breast

- ☐ Previous total mastectomy
- ☐ Previous partial mastectomy
- ☐ Previous reconstruction
- ☐ Other _____

RIGHT

Sub-cutaneous – RED
Intra-muscular – GREEN



Breast Lipografting Record

Lipografter_Record_UpdateApr2015V2.indd 1 19/05/2015 10:24 am

Appendix 4. Breast area record for lipografting left side: Version 2

Government of South Australia
SA Health

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Affix patient label here

Plastics and Reconstructive Surgery Department

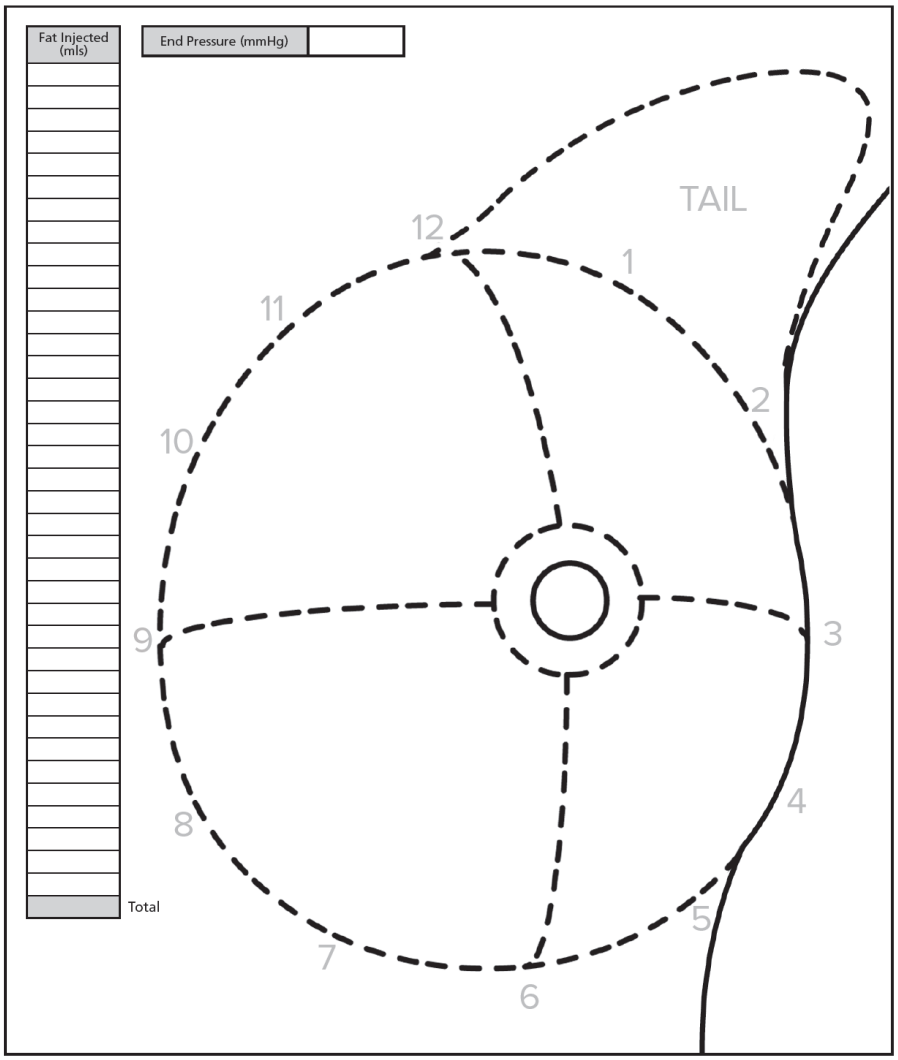
Breast Area Record For Lipografting

Left Breast

- ☐ Previous total mastectomy
- ☐ Previous partial mastectomy
- ☐ Previous reconstruction
- ☐ Other _____

LEFT

Sub-cutaneous – RED
Intra-muscular – GREEN



Breast Lipografting Record

Appendix 5. Brava device diary

Week 1-4.

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Time Started (Night)							
Time Finished (Morning – Tuesday am)							
Time (Total)							

Appendix 6. Skin care regimen

Skin Care Regimen

The following is a suggested skin care regimen if you begin to experience itchiness.

Morning:

Morning shower use Pinetarsol as directed on the box.

When completely dry after your morning shower, you can apply Sudacrem all over your chest area which can be left on whilst you have your breakfast. Sudacrem is for nappy rash and can be purchased in any chemist or supermarket in the baby section.

After approximately 30 mins apply Aveeno Baby cream all over your chest area and allow to absorb. Fragrance free soothing relief moisturising cream, it comes in a 140g tube (beige striped with a blue lid).

Night before BRAVA:

Shower (not getting your hair wet) approx. 2 hours before BRAVA application so the skin is completely dry (or a time frame enough to allow your skin to air dry).

Once the skin is dry apply Sudacrem all over the chest area.

When ready for bed/application of BRAVA, apply Aveeno Baby cream to the whole of your chest area and leave to absorb for 10 minutes.

If this doesn't solve the problem, also try:

A friction cream which can be found in Foodland and placed underneath

Tegaderm. (Gold Bond Friction Defence). This reduces friction between the skin and the adhesive.

The BRAVA should be able to be worn comfortably for 8-10 hours each night following this regime.

Another skin cream which may help is a over the counter cream called Silic 15.

Appendix 7 Participant information sheet BRAVA and AFG study

Government of South Australia
Southern Adelaide Health Service

FLINDERS MEDICAL CENTRE

Flinders Medical Centre

Bedford Park SA 5042

Department of Plastic and Reconstructive Surgery

PARTICIPANT INFORMATION SHEET

Pilot study of a breast expanding device and fat grafting for breast reconstruction after cancer.

Researchers:

Dr Benjamin Howes (PhD candidate). Dr Nicola Dean (Principle Supervisor of PhD candidate). Professor David Watson (Co-Supervisor of PhD candidate, Head of Surgery), Dr Beverley Fosh (Co-Supervisor of PhD candidate). Professor Steven Birrell (Head of the Breast and Endocrine Surgery Unit), Dr Jia Miin Yip (PhD-Determinants in Breast Reconstruction Outcome), Dr Pakan Kleinig (Consultant Radiologist), Mr Ruben Kannan (Consultant Plastic Surgeon)

Study Outline and Potential Benefits:

Currently available methods to restore the missing breast tissue after breast surgery are complicated. Current conventional surgeries have significant risks and involve long recovery times. This study is to investigate a new way of restoring lost breast tissue by using an external suction device (BRAVA device, see picture below) on the breast area combined with fat that is sucked (liposuction) from the thighs or

tummy. This way of reconstructing the lost breast tissue may work just as well as existing methods for reconstruction with less risks and recovery time. The study aims to test whether this method is easy for patients to tolerate, whether it works as well as other methods and whether the results are as good as conventional reconstructive surgery.

When looking at current success of the procedure, the literature suggests that some of the transplanted fat is resorbed/lost. With the BRAVA devices use in the USA, they are currently reporting only 20% graft resorption. This would be a promising result for fat grafting, and should result in good cosmetic outcomes, despite 20% resorption. We would like to confirm this outcome by measuring the amount of graft loss using a high quality 3D scanner and MRI.

The BRAVA device has two plastic domes that sit over the breasts and under your clothes. There is soft silicone at the base of the domes which is for comfort, but also to achieve a seal. There is gentle suction created by the device (20mmHg) which helps expand the breast over time (If worn as instructed). You will then have the liposuction procedure (under general anaesthetic) where we take fat from your tummy (or thighs) and place this fat into your breast to reshape the breast, in the space that has been created by the BRAVA device. The overall outcome is a return of your breast size and shape similar to what you had before breast cancer surgery.

Selection:

You were selected as suitable to participate in this study having been treated by the Surgical Oncologists at Flinders Medical Centre for breast cancer. You are considered to be a candidate for the new reconstructive procedure outlined in this form.

Aims of the Project:

To assess whether the use of a skin expansion device combined with fat grafting works for breast reconstruction and whether it is comfortable.

Summary of Procedures:

Enrolment into the study: At initial consultation we will explain the procedure, answer further questions that you have about the procedure and then formally consent for participation in the study.

Pre procedure

BRAVA device: Once you decide you would like the procedure you will try the BRAVA device. The BRAVA is worn in our Breast Clinic for 20 minutes, to see if it is comfortable. If you tolerate wearing the device we will give you a new device to take home. In order to prepare the breast for fat transfer (create room to graft), we ask that you wear the device for 10 hours per day (or overnight if you prefer), for four (4) weeks.

3D scan: You will have a 3D scan to assess the volume of your breast at this point.

Mammogram: If you have not had a mammogram within 6 months of your initial appointment, we will book you to have one in the Breast Unit. This is to ensure you are clear of breast cancer before starting this treatment.

MRI: MRI scan to assess volume of breast tissue as a comparison to 3D laser scan. This will be done the same day as your pre-op mammogram, and 3D laser scan (in most cases).

Procedure

BRAVA device: We ask that you use your BRAVA device for 48 hours non-stop before your procedure, taking it off once you meet with us the day of your operation.

Fat grafting: With BRAVA use your breast now has ample room for the fat transfer procedure. You will have fat grafting procedure under General Anaesthetic (GA) as a day procedure (you can go home the same day if there are no issues, eg pain or you are drowsy from the anaesthetic- we recommend you get picked up from the hospital).

Post Procedure

BRAVA device: We ask that you use the BRAVA device for the 48 hours after surgery, starting from when you get home (or in hospital so we can see if its tolerated). This will help the grafted fat in its new environment. After 48 hours we ask that you wear the BRAVA device for 10 hours per day until we see you in the outpatient department

3D scan: You will have a 3D scan at 3 months, 6 months and 12 months after you fat graft procedure. This will be to assess the volume achieved and maintained over time.

Mammogram: You will have a repeat mammogram at 6 months and 12 months post your procedure. After completion of the study period (12 months post fat grafting) you will have a consultation with a plastic surgeon (Dr Nicola Dean or Dr Quoc Lam). If you are not satisfied with the reconstructive results and you are identified as being more suitable for a more conventional surgical procedure, you will be listed to have this within 3 months of consultation (Category 2 on hospital waiting list).

<u>Commitments</u>:

As part of usual surgical follow up, we will see you in the Outpatient Department to monitor your progress. The initial appointment will be in the week after your procedure.

Three additional appointments will be made for you that will take 30 -45 minutes. This will be to measure the success of the fat graft procedure using a non-invasive 3D laser scanner (scanner similar to those used to scan barcodes at the grocery store) to measure breast volume, and stability of the fat graft. . Photographs (2D)

will be taken by our Clinical Photographer at the same time as the 3D scanner. This is a routine part of clinical care in this type of surgery because it helps the surgeon in the planning of your procedure and is a good reference for follow-up. These appointments will take 30-45 minutes and occur:

1) After BRAVA use prior to your fat grafting procedure

2) 3 months post fat graft procedure

3) 6 months post fat graft procedure.

At each of these appointments you will be asked to complete a BREAST-Q questionnaire which takes 10-15 minutes to fill out. It asks you questions about how you see the appearance of your breasts, physical well-being, psychosocial well-being, sexual well-being, and how you have found the treatment at our institution.

For research purposes, you will be required to attend additional appointments in the Radiology department to undergo a mammogram and a NON-contrast MRI scan before your procedure and 6 months post procedure. MRI is the gold standard for measurement, and would assist us in comparing this to our 3D laser scanner. This will take 10 minutes. You will also have a mammogram 12 months after the fat graft procedure for routine breast cancer follow up.

Benefits:

We hope that participation in this study will help you personally in improvement of your breast problem. In a wider sense, the results from this study will help us to decide whether it is wise to proceed with a larger study of breast expansion and fat grafting procedures. We hope that these new techniques will enable women in the future to have better results after breast cancer surgery with less invasive procedures.

Risks and Adverse Effects

The BRAVA device should be painless. If you find a 20 minute trial (at the hospital) to be comfortable, you will be given a BRAVA device to take home. Should you find it painful at any point you are advised to discontinue use and call for an appointment.

There are risks of bleeding and infection with any surgical procedure, but the risks with this particular procedure are low.

The pre-operative and 6 month mammogram you will require do carry a very small risk due to the radiation involved, similar to that of any X-ray. The dose of radiation is minimal, equivalent to a return plane trip to Melbourne. Each mammogram radiation dose is 0.18 mSV. We absorb natural background radiation at earth level of 2.4 mSV per year. The chance of 0.18 msV causing cancer in a life time is 4.5 in 1 million.

The MRI scan does not involve any risk to your health as long as you meet the criteria on MRI (eg. No metallic devices within your body, eg ear implants). If you do not meet the criteria this will not exclude you from the study. We will not be using contrast for the MRI.

The 3D scanner uses the same ‘laser’ that is used to scan barcodes at the supermarket, and to date, has not be found to cause harm to humans.

Compensation

There should be no additional cost incurred for you. The BRAVA device will be supplied to you free of charge. Should you live in the country, we will provide reasonable reimbursement for travel and accommodation. If you live locally, we will provide reimbursement for travel and parking (covering 4 visits to Flinders

Medical Centre). The Plastic and Reconstructive Surgery has received funding to cover the costs of this study, but is not involved in any commercial reimbursements for profit or financial gain. Participation in this study does not impact your basic legal right to seek compensation; however, if you do suffer harm, you may receive compensation without litigation.

Confidentiality

As per current hospital policy, all records containing personal information will remain confidential and no information which could lead to your identification will be released, except if required by law. Under Australian privacy law all information collected about you must be kept confidential. They may also be looked at by representatives of regulatory authorities and by authorised people from the hospital to check that the study is being carried out correctly. All these people have a duty of confidentiality to you as a research participant and no information that identifies you will be given to anyone else. If the results of this study are published, for example in scientific journals, you will not be identified by name. With your consent, your general practitioner will be informed of your participation in this study so that, if you need to see him/her for any reason, he/she will be aware you are involved in the study.

Publication

The project outcome will be published in scientific journals at a later date. This is to share information with the international medical profession for learning and progress purposes.

Withdrawal

Your participation in this study is entirely voluntary and you have the right to withdraw from the study at any time without giving a reason. If you decide not to participate in this study, or if you withdraw from the study, you may do so freely, without affecting the standard of care provided to you.

Invitation to Participate:

You are invited to participate in this research project but you do not have to be involved, whether you wish to or not is entirely up to you. Whether you take part or not, your medical care/relationship with the hospital will not be affected in any way. If you would like to participate, please telephone Dr. Ben Howes on 8204 2831 or 0419 393 230 and we will arrange an appointment to discuss this further and enroll you in the study if that is what you choose.

Contact

Dr Benjamin Howes (8204 2831), email howe0071@flinders.edu.au

Dr Nicola Dean (82045213), email Nicola.Dean@health.sa.gov.au

Andrea Smallman(Breast Care Nurse): 82047184, email Andrea.Smallman@health.sa.gov.au

Complaints

This study has been reviewed by the Southern Adelaide Clinical Human Research Ethics Committee. If you wish to discuss the study with someone not directly involved, in particular in relation to policies, your rights as a participant, or should you wish to make a confidential complaint, you may contact the Executive Officer on 8204 6453 or email research.ethics@health.sa.gov.au

Appendix 8. Pilot study of BRAVA and AFG consent form

Government of South Australia
Southern Adelaide Health Service

FLINDERS MEDICAL CENTRE

Flinders Medical Centre

Bedford Park SA 5042

Department of Plastic and Reconstructive Surgery

Consent to participation in research

Pilot study of a breast expanding device and fat grafting for breast reconstruction after cancer.

I, _____

(first or given names) (last name)

give consent to my involvement in the research project (BRAVA pilot study):

_____

I acknowledge the nature, purpose and contemplated effects of the research project, especially as far as they affect me, have been fully explained to my satisfaction by

_____

(first or given names) (last name)

and my consent is given voluntarily.

I acknowledge that the detail(s) of the following has/have been explained to me, including indications of risks; any discomfort involved; anticipation of length of time; and the frequency with which they will be performed:

1. Application of the BRAVA device pre and post fat grafting

2. Mammogram (Partial Mastectomy): Pre procedure (Unless already done within 6 months) and 6 months post procedure

3. Photographs to be taken pre-BRAVA, before surgery and at 3, 6 and 12 months following surgery

4. Laser body scan before BRAVA, pre-op, 3, 6 and 12 months following surgery.

5. MRI scanning (non- contrast): Pre surgery and 6 months post-surgery

6. Completion of surveys before surgery and at 3, 6, and 12 months following surgery (each questionnaire takes 10-15 minutes)

7. Access to your medical records and inclusion into research via data collection

I have understood and am satisfied with the explanations that I have been given.

I have been provided with a written information sheet.

I understand that my involvement in this research project may not be of any direct benefit to me and that I may withdraw my consent at any stage without affecting my rights or the responsibilities of the researchers in any respect.

I declare that I am over the age of 18 years.

I acknowledge that I have been informed that should I receive an injury as a result of taking part in this study, I can approach an

independent person in the hospital for advice regarding legal action and determine whether I should be paid.

Signature of Research Participant: _____

Date: _____

I,_ have described to _

the research project and nature and effects of procedure(s) involved. In my opinion he/she understands the explanation and has freely given his/her consent.

Signature: _____

Date: _____

Status in Project:_____

Appendix 9. Traditional breast reconstruction times

Expander implants

Operation	Number of Patients	Average Procedure Time
Expander Insertion (Unilateral)	4	93
Expander Insertion (Bilateral)	8	125
Exchange of expander for implant (Unilateral)	15	86
Exchange of expander for implant (Bilateral)	47	129
Exchange of expander for implant and contralateral mastopexy	12	154
Unilateral mastectomy + expander insertion	6	148
Bilateral mastectomy + bilateral expander insertion	7	214

Latissimus Dorsi Flap Reconstruction

Operation	Number of Patients	Average Procedure Time
Unilateral	7	341
Unilateral LD + contralateral mastopexy	4	423
Unilateral LD + expanders	7	299
Unilateral mastectomy and unilateral lat dorsi	3	334
Unilateral mastectomy and bilateral lat dorsi	1	504
Unilateral mastectomy and unilateral lat dorsi + insertion of tissue expander	12	374
Latissimus Dorsi Reconstruction Alone (Bilateral)	0	
Unilateral mastectomy with bilateral LD + Expanders	5	658.9
Bilateral LD + Expanders	3	481.4
Bilateral Mastectomy and Bilateral LD alone	2	681.9
Bilateral Mastectomy, unilateral LD +expanders, unilateral Expanders	1	395
Bilateral Mx + LD+ expanders	2	678.6

Free Transverse Rectus Abdominus Myocutaneous Flap Reconstruction

Operation	Number of Patients	Average Procedure Time
Unilateral TRAM	14	496
Bilateral TRAM	1	650
Unilateral mastectomy and Free Transverse Rectus Abdominus	7	571

Appendix 10. Study participant information sheet and consent form co-culture study

Government of South Australia
Southern Adelaide Health Service

FLINDERS MEDICAL CENTRE

Department of Plastic and Reconstructive Surgery

Flinders Medical Centre

Bedford Park SA 5042

Participant Information Sheet/Consent Form

Interventional Study - *Adult providing own consent*

Flinders Medical Centre

Title: **Does fat grafting influence risk of breast cancer? An *in vitro* study of adipose tissue and cultured breast cells.**

Short Title: Does fat grafting influence risk of breast cancer?

Protocol Number: 355.14

Project Sponsor: None

Coordinating Principal Investigator/Principal Investigator: Dr Nicola Dean.

Associate Investigators: Professor David Watson, Dr Damian Hussey, Dr Beverley Fosh,

Dr Benjamin Howes.

Location: Flinders Medical Centre/Flinders University.

Part 1 What does my participation involve?

1 Introduction

You were selected as suitable to participate in this study as you are undergoing an elective operation at Flinders Medical Centre. The elective procedure and tests involved in this research are approved by the Australian Federal Government. This study will be undertaken in a laboratory setting.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

• Understand what you have read

• Consent to take part in the research project

• Consent to have the tests and treatments that are described

• Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

Aim

The purpose is to establish whether fat transfer creates a more dangerous environment for breast cells, increasing risk of malignant growth and transformation.

Significance

Breast cancer surgery has long term effects on women's quality of life because the defect left from the surgery can lead to reduced self-esteem, diminished psychological well-being, a feeling of "imbalance" and restricted freedom of dress. Currently available methods to restore the missing breast tissue are complicated, have significant risks and involve long recovery times.

Fat grafting is increasing in popularity as an alternative to conventional breast reconstruction procedures following breast cancer surgery. Results of the proposed study aims to settle the debate regarding the oncological risk of fat grafting procedure in women who have had breast cancer surgery, by establishing the level of risk associated with this procedure. Should its safety be confirmed, this would mean significant benefits both for patients and the Health Care system in terms of drastically shortened operating time, reduced problems during your operation, shorten hospital stay and therefore increase the number of breast reconstructions.

Mastectomy affects women's quality of life owing to disfigurement. Breast reconstructive procedures are increasingly sought by mastectomy patients to improve aesthetics and psychosocial impact of breast surgery. Current surgeries can be quite invasive and associated with long recovery times. In addition, some women do not like the idea of reconstruction using an artificial implant. Autologous fat (from the

same individual) could be an ideal material for breast reconstruction as it is readily available (liposuction from abdomen/inner/outer thigh), can be collected with minimal problems and as it is from the same individual there is no risk of rejection. Fat transfer is performed using small needles and cannulae and therefore leaves only tiny scars and involves no interference with muscles, nerves or blood vessels. For these reasons, autologous fat transfer is gaining popularity for breast reconstructive procedures. Once fat is taken from the donor site it is transplanted to the breast after excess fluid (local anaesthetic and blood) is decanted off. At this point, the fat is ready to graft. The fat graft is then deposited into the recipient site (breast area). The fat that is grafted to the recipient site does not have its own blood supply and relies on the surrounding tissue to supply it with nutrients. Currently there is controversy surrounding the use of concentrated lipo-aspirated fat in women who have previously had breast cancer. The laboratory and human studies have conflicting information regarding cancer risk. There are no clinical studies which show an increase in cancer formation from lipo-aspirated fat. One clinical study by Petit et al. involving fat grafting and lipoaspirate specifically outlines a need for more pre-clinical research into the paracrine effects of fat and breast cell lines.

Additional Project Information

The results of this research will be used by the study doctor Dr Benjamin Howes to obtain a Doctor of Philosophy degree.

This research has been initiated by the study doctor, Dr Benjamin Howes/Dr Nicola Dean

This research is also being conducted at The Hanson Institute, Adelaide University.

3 What does participation in this research involve?

Enrolment into the study: At initial consultation we will explain the process of fat sampling, answer further questions that you have about the procedure and then formally consent for participation in the study. Enrolment will occur during pre-admission clinic and is entirely voluntary.

Procedure

Fat sampling: During your elective procedure, subcutaneous fat (fat beneath the skin surface) is usually exposed during your operation. Either a small sample of this fat will be taken from this layer, or will be taken from part of the fat that would have been discarded as a by-product of the operation. This amount of fat sampling is negligible and in no way will affect the outcome of your surgery.

Post Procedure

Elective Surgery: Your post-operative recovery will depend on which elective surgery you have undergone. What to expect in the post-operative period can be discussed with your surgeon. The fat and blood sampling will not affect the follow up and appointments which would have otherwise been made as standard procedure.

Randomisation and Control Group:

There will not be randomisation. Fat will be used in the laboratory on normal breast cells and breast cancer cells. You will be participating in a cross-over study. In a cross-over study your adipose tissue will be used on different types of breast cells in order to see the effect fat has on growth and proliferation.

Duration of Research Projects

In the initial 4 months of the project Study 1 is likely to yield recruitment of between 4-6

patients. After which, there will be a 1 month period of write up and refinement of techniques, prior to the commencement of Study 2. At this stage the results of Study 1 will be written up for presentation and publication in a peer-reviewed journal. Study 2 will be carried out in the following 7 months, with sample collection taking place in the first 5 of these months. Completion of culture, collection of results and analysis will take place in the final 2 months.

What do I have to do?

There are no specific lifestyle restrictions e.g. physical restrictions, participation in sport

or dietary restrictions associated with this research. This research project does not require medication changes. You can still donate blood.

Research Plan

There will be two studies within this research. Study 1 will use a normal breast cell line (MCF10A) to observe the effect of lipoaspirate on a normal breast cell line. Study 2 will use different areas of fat taken from other parts of the body and the effect observed on breast cancer cell lines MCF7 and non-malignant breast cell lines MCF10A.

Study 1

Harvested fat graft (lipoaspirate) + non-malignant breast cells (MCF10A)

Study 2

- Harvested fat graft (from previous recipients) + non-malignant (MCF10A) and malignant breast cells (MCF7)

- Fat from vascularised flap (from previous reconstruction) + non-malignant (MCF10A) and malignant breast cells (MCF7)
- Fat from other areas of the body + non-malignant (MCF10A) and malignant breast cells (MCF7)

Patients undergoing fat grafting will be asked if a sample of fat can be used for the research. Patients wishing to be involved in the study will give their informed consent regarding the use of a fat sample and collection of a blood sample before the procedure. For Study 2 patients undergoing other surgery where sub-cutaneous fat is accessed (such as abdominoplasty or laparotomy) will be approached for consent to donate a specimen of fat. There is likely to be 4-6 patients in each group.

Patients will be recruited from Flinders Medical Centre. Laboratory studies will also be conducted within Flinders Medical Centre through the Department of Surgery. Some laboratory input regarding collaboration will be done with The Hanson Institute at The University of Adelaide. They have previous experience with this type of cell culturing.

This research project has been designed to make sure the researchers interpret the results in a fair and appropriate way and avoids study doctors or participants jumping to conclusions. Your adipose tissue will not be identifiable as yours. You will be allocated a study number to reduce investigator bias prior to data analysis

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with Flinders Medical Centre.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research; however, possible benefits may include better understanding of the use of the Autologous Fat Grafting procedure for breast reconstruction in women who are being treated post breast cancer surgery.

There will be no clear direct benefit to you from your participation in this research. But rather results will help understanding regarding the effect of fat on breast cells.

9 What are the possible risks and disadvantages of taking part?

Risks and Adverse Effects

There are risks of bleeding and infection with any surgical procedure, but the risks with this particular procedure are low. The sampling of fat may come from tissue that would have been discarded during your elective operation. Otherwise, the small sample should not result in increased bleeding or infection risk. Should you have a post-operative wound infection, this will be treated with either oral or intravenous antibiotics according to your needs..

The blood sample will be taken from an established intravenous line that would have otherwise been placed for the purpose of administering the general anaesthetic. There is therefore unlikely to be any increased risk or adverse effects.

This research is unrelated to your current procedure and is unlikely to uncover a medical condition of which you are unaware.

If you become upset or distressed as a result of your participation in the research, the study doctor will be able to arrange for counselling or other appropriate support. Any counselling or support will be provided by qualified staff who are not members of the research project team. This counselling will be provided free of charge.

10 What will happen to my test samples?

The collection of fat and blood, as described, is a mandatory component of this research only. The fat will be used immediately for cell culturing. The blood sample will also be stored for blood level testing of leptin and adiponectin. These will be stored in accordance with protocols previously established within the Department of Surgery/Flinders University laboratory. Both fat and blood will be used for this study purpose. Fat will be placed with breast cells to see whether or not they potentiate proliferation or mutation. All samples will be destroyed at the end of the research study which is likely to be within a 12 month time frame. Samples will be identifiable to the research doctor only. Otherwise, data collected will not be re-identifiable.

You will be asked to provide additional consent for the collection of your blood and tissue during the research project.

The proposed blood tests may include a screening test for HIV (also called the 'AIDS' virus) and Hepatitis. This is because the study doctors may need to know your HIV status in the event of a needle stick injury. You will receive information and counselling before the test. If a test shows you have HIV or Hepatitis, you will have follow-up counselling and medical advice. If your test results are positive, the

study doctors are required by law to notify government health authorities. Signing the consent form means that you agree to have this testing; it will not be done without your consent.

12 Can I have other treatments during this research project?

Whilst you are participating in this research project, you may take all of the medications or treatments you have been taking for your condition or for other reasons.

13 What if I withdraw from this research project?

If you decide to withdraw from the project, please notify a member of the research team before you withdraw.

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected by the sponsor up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you join the research project.

14 Are there any cost associated with participation?

There are no additional costs associated with participating in this research project, nor will you be paid. All tests and medical care required as part of the research project will be provided to you free of charge.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

As per current hospital policy, all records containing personal information will remain confidential and no information which could lead to your identification will be released, except if required by law. In accordance with Australian privacy and other relevant laws, you have the right to have all information collected about you remain confidential. They may also be looked at by representatives of regulatory authorities and by authorised people from the hospital to check that the study is being carried out correctly. All these people have a duty of confidentiality to you as a research participant and no information that identifies you will be given to anyone else. If the results of this study are published, for example in scientific journals, you will not be identified by name.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission. Your name or date of birth will not be used in the published material. The main focus will be regarding the data obtained rather than patient specifics.

Information about your participation in this research project may be recorded in your health records.

In accordance with relevant South Australian privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital. Should you require compensation not covered by insurance arrangements, you can initiate action through the Courts.

18 Who is organising and funding the research?

This research project is being conducted by Dr Benjamin Howes, Dr Beverley Fosh, Dr Nicola Dean, Professor David Watson and Dr Damian Hussey.

No member of the research team will receive a personal financial benefit from your involvement in this research project (other than their ordinary wages). This research itself (consumables etc.) has been funded by Flinders University Department of Medicine through Research Higher Degree student maintenance.

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Southern Adelaide Clinical Human Research Ethics Committee. The

ethical aspects of this research project have been approved by the HREC of Flinders Medical Centre.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

Standards of care will be supervised by the Flinders University.

20 Further information and who to contact

Clinical contact person

Name	Dr Benjamin Howes
Position	Research Registrar/PhD Candidate Plastic & Reconstructive Surgery
Telephone	82042803
Email	howe0071@flinders.edu.au

Name	Andrea Smallman
Position	Breast Care Nurse Plastic & Reconstructive Surgery
Telephone	82047184
Email	Andrea.Smallman@health.sa.gov.au

Name	Dr Nicola Dean
Position	Consultant Surgeon, Plastic & Reconstructive Surgery
Telephone	82045213
Email	Nicola.Dean@health.sa.gov.au

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

Complaints contact person

Name	Bev Stewart Campbell
Position	Research Governance Officer
Telephone	8204 4507
Email	bev.stewart-campbell @health.sa.gov.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Consent Form - *Adult providing own consent*

Title: Does fat grafting influence risk of breast cancer? An in vitro study of adipose tissue and cultured breast cells.

Short Title: Does fat grafting influence risk of breast cancer?

Protocol Number: 355.14

Co-ordinating Principal Investigator: Dr Nicola Dean.

Associate Investigators: Professor David Watson, Dr Beverley Fosh, Dr Damian Hussey, Dr Benjamin Howes.

Location: Flinders Medical Centre/Flinders University.

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Flinders University concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential. I have had an opportunity to ask questions and I am satisfied with the answers I have received. I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care. I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signaturere _____ Date _____

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/

Senior Researcher† (please print)

Signature _____ Date _____

† A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

I consent to the storage and use of blood and tissue samples taken from me for use, as described in the relevant section of the Participant Information Sheet, for:

- This specific research project

Name of Participant (please print) _____

Signature _____ Date _____

Name of Study Doctor/

Senior Researcher† (please print)

Signaturesure _____ Date _____

† A senior member of the research team must provide the explanation of and information concerning the research project.

Note: All parties signing the consent section must date their own signature

Appendix 11. Disposables and other equipment

Interpath Service PTY LTD: Unit 1/46 Sheehan Road, PO Box 340, Heidelberg West VIC 3081, sales@interpath.com.au

Greiner 10ml serological pipette (607180)	pack of 200
Greiner 5ml serological pipette (606180)	pack of 200
Greiner 25ml serological pipette (760180)	pack of 200
Greiner 75 tissue culture flask (658175V)	pack of 120
Greiner 25 tissue culture flask (690175V)	pack of 200
Falcon 50ml tube (227261S)	pack of 500
Corning 6 Well TC Plate with lid (657160)	pack of 100
Corning 96 Well TC Plate with lid (655180)	pack of 100
Corning 24 Well TC Plate with lid (662160)	pack of 100
200µl Filter Tip (24700)	pack of 100
250µl Filter Tip (24900),	pack of 80
100µl Filter Tip (24600),	pack of 100
20µl Filter Tip (24500),	pack of 100
Eppendorf 1.5ml Centrifuge Tube (EPP0030-125-150)	1,000
Eppendorf 2ml Safelock tubes (EPP0030-120-094)	1,000
Eppendorf 1.5ml Safelock tubes (EPP0030-120-086)	1,000

Life technologies

DPBS (73 14190250)	10x 500 mL

Dulbecco's Modified Eagles Medium (DMED) with Ham's F12 (1:1 ratio) and HEPES

α-Modified Eagles Media 5 x 500mls

Penicillin Streptomycin Solution (75 15070063)	100 mL
Trypsin .5% (82 15400054)	100 mL
Foetal bovine serum (10099141)	500mL
Trypan Blue Stain (T8154)	100mL

Appendix 12. Differentiation media recipe

Product	Molecular Weight	Final Concentration	In 200mls	Final stock solution
FBS		3%	6mls	
IBMX	222.2	200nm	11.11mg	
Biotin	244.31	66 µM	3.225mg	66 mM in 1M sodium hydroxide
D-pantothenate	238.27	34µM	1.62mg	34mM dissolved in water
Rosiglitazone	357.426	5µM	0.3574	5 mM dissolved in DMSO
Dexamethasone	392.4	1µM	0.07848	1 mM in 10mm ETOH
Human Insulin	5807.57	200nM	0.2323	200µM dissolved in PBS
DMEM/Hams F12			194mls	

Appendix 13. Cultrex® Growth Medium

Cultrex® requires its own medium when populated with cells for culture. The ingredients were supplied by Invitro Technologies® and were mixed to 200ml volume. The bottle was labelled Cultrex® growth medium and was stored in a refrigerator at a temperature of 4^0C. The other ingredients for the medium were made separately and added in aliquots to the 200ml volume of medium.

1. Cultrex® medium preparation
1. Thaw 3-D Cultrex® Matrix™ reduced growth factor basement membrane at 2-8 °C overnight (Debnath et al., 2003).
2. Once thawed, work on ice to prevent increased temperature leading to solidification of the Cultrex® .
3. Cultrex® growth media (98mLs)
4. Place 3-D Culture Matrix™ RGF BME (2mls for final concentration of 2%) in a sterile container.

Preparation of Cultrex® -three-dimensional growth matrix

In order to prepare the well plates to culture breast cells in conditioned media provided from fat from different parts of the body, the Cultrex® three-dimensional growth matrix was prepared as instructed by the provider (Invitro Technologies®).

1. Thaw the 3-D Culture Matrix™ RGF BME at 2-8 °C overnight (Debnath et al., 2003).
2. Once thawed, work on ice to prevent an increased temperature leading to solidification.
3. Add 250 µL of 3-D Culture Matrix™ RGF BME per well in a sterile 48-well plate.

4. Incubate the plate at 37 °C for 30 minutes to promote the gelling of the matrix.
5. Harvest cells from the culture and dilute the cells to 1 x 10^4 cells/mL in 24 mL of assay medium.
6. Add 500 µL of cell suspension to each well of the 48-well plate containing 3-D Culture Matrix™ RGF BME.
7. Incubate the plate at 37 °C in the incubator overnight.
8. Each day, observe the cell growth and structure formation via microscope, and place the 48 wells back into the incubator overnight at 37 °C.
9. On day 4, carefully pipet off the old media using a sterile serological pipette and replace it with new assay media. Repeat on day 8 and day 12.
10. When the structures have grown to the desired size, prepare the cells for analysis.

Any unused 3-D Culture Matrix™ RGF BME can be stored at 4 °C for up to 1 week or stored in working aliquots at –20 °C in a manual defrost freezer.

Appendix 14. Immunofluorescence recipe and procedure

Immunofluorescent staining of MCF10a breast cells in three-dimensional culture This protocol describes how to immunostain MCF10a cells in a three-dimensional matrix of collagen and Cultrex®.

1. Methanol, Acetone, PBS: Glycine
1. Permeabilisation buffer: 0.5%
2. Triton X-100 in PBS
3. Immunofluorescence wash
4. Primary Block: 1X IF 10% Goat serum
5. Secondary Block: 1X IF 10% Goat serum + 1:100 dilution of $F(Ab)_2$
6. 10% Formalin: Sigma Catalog # .HT50-1-4.
7. Dilute 1:1 in 1xPBS to make a 5% solution.
8. Goat Serum: Sigmal Cat# G 6767
9. F(ab)2: Jackson Immunochemicals Catalog # M 35200.
10. Secondary antibodies: Invitrogen
11. TOPRO-3: Invitrogen
12. DAPI: Boehringer Manheim Catalog # 236 276
13. ProLong Anti-Fade: Invitrogen Catalog # P-7481

Immunofluorescence procedure

All aspirations were performed with mild suction to avoid disruption of the Matrigel and acini attachment.

1. Aspirate the media andwash 2 times with 1X PBS.
2. Fixation: incubate structures with 5% Formalin (dilute 10% stock 1:1 in PBS) for

30 minutes at room temperature (RT) or use 1:1 mix of methanol:acetone for 10 minutes at -20 ^{o}C. Rinse 3 times, 10 minutes each with PBS:Glycine.

3. Permeabilisation: if fixing with formalin, permeabilise using 0.5% Triton X-100 in PBS at RT for 5 minutes. Rinse 3 times, 10 minutes each with 1XIF wash at RT.
4. Primary Block: incubate with 200 µL of primary block for 1-1.5 hours at RT.
5. Secondary Block: aspirate the primary block and incubate with 100 µL of secondary block for 30-40 minutes at RT.
6. Primary antibody: aspirate the secondary block and incubate with 150 µL of primary antibody diluted in secondary block for 1-2 hours at RT on a gentle rocker. If the structures were fixed with formalin, it is possible to incubate the primary antibody at RT or 4 ^{o}C. Note: Incubation at 4 ^{o}C could liquefy the Matrigel resulting in loss of the structures!
7. Rinse 3 times for 20 minutes each with 1X IF at RT.
8. Secondary antibody: incubate with 150 µL of secondary antibody diluted in a primary block for 50-60 minutes at RT. Almost all of our secondary antibodies were Alexa-conjugated from Invitrogen-Molecular Probes and were used at 1:500 dilution. From this point on, the experiment proceeds in darkness (wrapped in aluminium foil).
9. Rinse 3 times, 20 minutes each with 1X IF wash at RT.
10. (Optional Step) Nuclear Co-Stain: Incubate with 1X PBS containing 5 µM TOPRO-3 for 15 minutes at RT.
11. Nuclear Co-Stain: incubate with 1XPBS containing 0.5 ng/mL DAPI for 5-10 minutes at RT. Mount the slides with freshly prepared Prolong Anti-Fade reagent and allow to dry overnight at RT. Once dry, the slides can be stored at RT for a few weeks or at -20 ^{o}C for two months.

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