Characterisation of Fibre–Forming Peptides and Proteins by Means of Atomic Force Microscopy

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Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or 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.

Rhiannon G. Creasey

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Abstract

Atomic force microscopy (AFM) is a high–resolution microscopic technique highly suitable for investigating biological entities. Chapter 1 reviews the use of AFM for investigating fibre–forming peptides and proteins, followed by the application of AFM to peptide–based dendrimers in chapter 2, fungus–based proteins in chapter 3 and whole human tissue in chapter 4. This investigation is supported by more traditional analytical techniques such as optical, electron and fluorescence microscopy, dynamic light scattering and circular dichroism spectroscopy.

In chapter 2, the aggregation properties of peptide–based dendrons and dendrimers were investigated using AFM. 3rd– and 4th–generation dendrons made from L–lysine showed gelation via a unique vesicle–driven pathway, confirmed by transmission electron microscopy, forming a dense network of nanofibres. The symmetrical dendrimers also formed nanofibre–based gels, which could be polymerised using UV irradiation to form tightly–packed gels with altered optical, Raman and fluorescence properties. UV irradiation through a photomask allowed the generation of crosslinked gel patterns. Gels from dendrons and dendrimers may be suitable for use in biomaterial applications for cell seeding assays, tissue engineering, or for drug delivery.

Chapter 3 dealt with the aggregation of fungal proteins. The recent identification of genes encoding three arabinogalactan–like (AGL) proteins of the fungi *G. intraradices* suggests that AGL proteins may be involved in the formation of the symbiotic interface between a common fungus and plant roots. Currently, the nature of cell wall modifications in this interface is unknown. Here, AFM was applied to investigate the self–assembly of the fungal proteins rAGL1 and rAGL3 and the growth of nanofibres and microtubules was

observed and described. Peptides based on the repeat regions seen in the AGL sequences were also observed to form fibres as seen by AFM and optical microscopy. The secondary structure of the proteins and peptides – hypothesised to be responsible for creating the interface of root apoplasts and fungi – were found to be primarily disordered or polyproline II helices by circular dichroism spectroscopy. Understanding of the structural properties of these proteins is vital to the process of *G. intraradices* symbiosis. Self–assembling peptides based on these proteins may find applications as innovative self–assembling biomaterials.

Protein aggregation is of significant interest to various disciplines including ophthalmology. One ocular disease hallmarked by protein aggregation is known as pseudoexfoliation (PEX) syndrome. This condition is caused by the formation of insoluble aggregates in the eye, and is clinically characterised by the deposition of proteinaceous material on the anterior lens capsule. The ultrastructure of PEX material is poorly characterised, despite numerous proteomic and genomic studies. The novel application of AFM–based antibody recognition imaging is applied in chapter 4 for determination of the molecular nature of PEX material on lens capsules in their native state. Topographical AFM images and antibody recognition images were obtained simultaneously to determine the specific location of clusterin, lysyl oxidase–like 1, and elastin proteins in and around PEX aggregates using antibody–modified AFM probes. Multiple AFM–based techniques were tested, and TREC was found to be the most suitable technique for recognition on whole unprocessed tissue samples. Future studies into AFM–antibody recognition techniques, such as quantitative nanomechanical mapping, may lead to interesting data combinations of mechanical and compositional information.

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List of Abbreviations

Abbreviation	Expansion
αS	Alpha–Synuclein
AFM	Atomic Force Microscope/y
AGL	Arabinogalactan–Like
AM	Arbuscular Mycorrhizal
APTES	3–Aminopropyltriethoxysilane
Aβ	Beta–Amyloid
CD	Circular Dichroism
CM	Contact Mode
CR	Congo Red
DCC	Dicyclohexylcarbodiimide
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic Acid
E. Coli	Escherichia Coli
EM	Electron Microscope/y
G. Intraradices	Glomus Intraradices
HOPG	Highly Ordered Pyrolytic Graphite
hr	Hour
LOX	Lysyl–Oxidase Like
min	Minute
MS	Mass Spectrometry
NHS	N–hydroxysuccinimide
PBS	Phosphate Buffered Saline
PDA	Polydiacetylene
PEG	Polyethylene Glycol
PEX	Pseudoexfoliation
PPII	Polyproline II
PrP	Prion Protein
QNM	Quantitative Nanomechanical Mapping
SEM	Scanning Electron Microscope/y
SPM	Scanning Probe Microscope/y
TEA	Triethylamine
TEM	Transmission Electron Microscope/y
ThT	Thioflavin T
TM	Tapping mode
TMEDA	N,N,N0,N0–tetramethylethylenediamine
TREC	Topography and Recognition imaging
UV	Ultraviolet
Z	Benzyloxycarbonyl group