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Protein superstructures are of great interest to various disciplines; they can be the cause of debilitating diseases, or the foundation of advanced nanomaterials. The goal of this project is to apply nano-scale techniques such as atomic force microscopy (AFM) to the characterisation of such protein interactions in their native environment. For this purpose, an ocular disease hallmarked by protein aggregation known as Pseudoexfoliation syndrome (PEX) was used as a proof-of-concept for the application of AFM-based antibody recognition imaging. This technique utilises an AFM probe as a biosensor for a specific protein, and obtains high resolution 3-dimensional topographical images of a surface whilst simultaneously measuring recognition of the protein of interest.

For the first time, AFM antibody recognition imaging was applied to whole, untreated tissues. A travel grant provided by ASBTE allowed a lab visit to world leaders in AFM antibody recognition imaging and AFM tip modification, Dr Peter Hinterdorfer and Associate Professor Hermann Gruber, where clusterin was detected at the nano-scale on human lens capsule samples. Using the methodologies developed during this lab visit, further studies can now be done on this tissue to determine the molecular interactions of the protein aggregates involved in PEX. The implications of this short lab visit for future research in biomaterials, tissue formation, clinical diagnoses, and many more biological investigations are staggering, and this technique is certain to be utilised for many studies to come. Keep an eye out for the publication that is to follow this work.

Travel outcomes

Protein aggregation is a significant clinical issue as it can cause a range of diseases, from Alzheimer's disease to type II diabetes and cataracts. Such protein structures can also form a wide variety of natural and artificial structures and materials. Amyloid fibres are already being explored as an advanced nanofibre material; further protein-based investigations could lead to biocompatible materials with the strength and versatility to be used for implant devices, drug delivery agents, cell therapy approaches, and much more. One disease hallmarked by protein aggregation is Pseudoexfoliation syndrome (PEX); this condition is caused by the formation of insoluble protein aggregates in the anterior segment of the eye.

Recently, we and other groups have determined the molecular nature of PEX by proteomic and immunohistochemical analyses. As PEX is a complex aggregation of proteins, the structure at the molecular level is poorly understood. This project addresses the issue of characterising the molecular nature of PEX aggregates on lens capsules in their native state by atomic force microscopy (AFM) based antibody recognition imaging. We collaborated with the laboratory which has pioneered this technique and applied it for the first time to tissue analysis to detect and image the PEX aggregates in their native state on the lens capsule. Three antibodies had been planned for this work; anti-clusterin, anti-LOXL1, and anti-apolipoprotein E as these proteins have been implicated in the PEX pathophysiology. However, due to various limitations (the most prominent of which was time) the only protein investigated was clusterin. This versatile protein is known to be a chaperone molecule; it also has a variety of extracellular functions, many of which aren’t yet understood. This protein

became the model system upon which proof-of-concept experiments were run, as it is the most convenient to obtain commercially.

Initially, force spectroscopy was used to confirm the probe modification using anti-clusterin against a surface to which clusterin was bound. Several surface chemistries were trialled to ensure the protein was not mobile; eventually, it was proven that the probe tip bound specifically to the protein and could be blocked by the introduction of free proteins.

The modified probes were then used in a picoTREC AFM system on control lens capsules without the PEX disease. Topography was observed in unison with recognition, and was able to be removed from the images using various methods such as the introduction of free protein to block the antibody. Once clusterin was shown to be present on normal lens capsules, the technique was applied to PEX-affected lens capsules. Some areas of the aggregates were too large to be imaged using the AFM, however smaller aggregates were successfully imaged using both functionalised and non functionalised probes. Clusterin was detected on the surface of the lens capsule and amongst the aggregates – this evidence further implicates this protein in the pathophysiology of PEX. If the chaperone nature of clusterin is the cause of the fibres created in PEX, it could potentially be used for self-assembled fibres of a strong, insoluble and biological nature. With further development, the methodologies which were learnt in Europe are suitable for analysing protein aggregates in a physiological environment. These structures can provide insight to new material development and protein interactions in the body.

A secondary outcome of this project was to reach a more profound understanding of the pathophysiological basis for PEX, so that methods can be developed to screen those at risk of developing PEX-associated glaucoma and losing vision. Improved understanding of the disease can also lead to the development of preventative and treatment strategies in the future. This aim was not achieved; however the proof-of-concept for this technique now means that it will be applied to furthering this aim in the future.

Funding was provided for travel to the University of Linz in Austria to work with the world leaders in AFM antibody recognition imaging and AFM tip modification, Dr Peter Hinterdorfer and Associate Professor Hermann Gruber. A publication is expected following this travel, and is currently being drafted to distribute this technique proof-of-concept and the results obtained from it. ASBTE is to be acknowledged as a funding source for this publication.