

## Raman spectroscopic evaluation of age-related changes in Bruch's Membrane

J.J. McGarvey<sup>1A</sup> ( [j.mcgarvey@qub.ac.uk](mailto:j.mcgarvey@qub.ac.uk) )

J.R. Beattie<sup>1B</sup>, A.M. Pawlak<sup>1B</sup>, M.E. Boulton<sup>2</sup>, A.W. Stitt<sup>1B</sup>.

<sup>A</sup>School of Chem & Chem Engineering, <sup>B</sup>Centre for Vision and Vascular Science, <sup>1</sup>Queen's University Belfast, Belfast, United Kingdom; <sup>2</sup>Anatomy and Cell Biology, University of Florida, Gainesville, FL.

## PURPOSE

- Bruch's membrane (BM) is a highly dynamic matrix that is integral to RPE and outer retinal function. *Reactive intermediates of metabolism are elevated during ageing and the adducts formed can modify the collagens of BM and this has a critical role in the development of AMD.*

Analysing advanced glycation / lipoxidation endproducts (AGEs/ALEs) normally requires invasive or destructive & highly specific analyses.

The purpose of this study is to investigate the potential Of the non-invasive technique of Raman spectroscopy to **multiplex** age-related biochemical information.

## SOME BACKGROUND ON RAMAN

### Raman Microscopy and Biomedicine – Why?

- high information level:
- chemical
- physical
- spatial
- Water is a weak Raman scatterer
- non contact, focused radiation (UV, Vis, NIR)
- Raman scattering can be investigated under non-invasive conditions

## Raman microscopy?

- Raman microscopy adds spatial to spectral resolution and also opens up the possibility of mapping biological samples for chemical and physical composition at a cellular level (**and unlabelled**).
- This spatial resolution is important as biological samples are often inherently heterogeneous with biochemical distributions that may differ from sample to sample and also from point to point within the same sample.

## Some important points about biological tissue

- Raman spectra of biological materials are a complex mixture of constituents (*>50 unique constituents are not uncommon*)
- It is often difficult to get an exact matching reference material
- Interactions of a sample with its matrix may influence its Raman signal (e.g. *hydrophobic/hydrophilic interaction in proteins*)
- Statistical approaches become essential (multivariate analysis) e.g. *principal component analysis (PCA)*

## DATA ANALYSIS

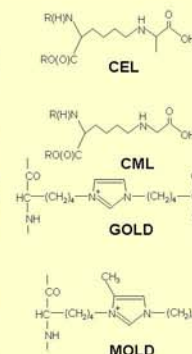
By extracting the signals from the PCA and testing them against the spectral database of biochemicals we identified the AGE/ALE adducts that were detectable in the Raman signals recorded from donor BM tissues.

## METHODS

- BM from human post-mortem eyes were flat mounted and probed using a Raman microscope. Area probed : 100 x 100  $\mu\text{m}^2$
- Confocal Raman microscope: nominal 1.0  $\mu\text{m}$  axial resolution, 0.8  $\mu\text{m}$  XY resolution
- Excitation wavelength : 633 nm excitation (up to 100 mW); x 100 objective
- 3 repeat scans on each sample (N = 56)
- A spectral database of a wide range of collagens, fats, porphyrins and metabolic adducts was recorded.
- The database covered most AGE/ALEs known to occur in human tissues.

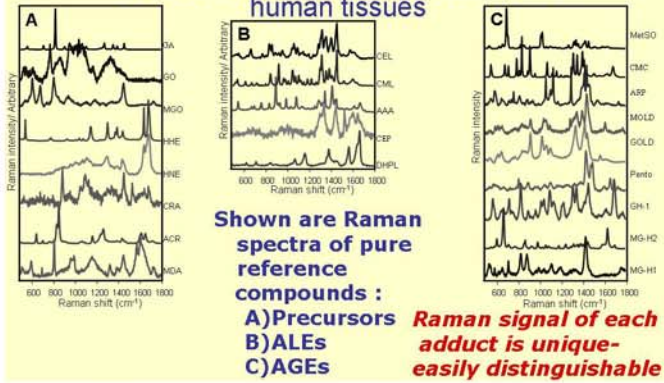
## RESULTS (I)

- The Raman spectra of a number of different classes of AGE/ALEs are displayed in next slide (→)
- Despite close structural similarities in some instances, the recorded AGE/ALEs show distinctly different spectra ( *'fingerprints'* ) that allow even the most similar structures to be distinguished.
- Thus, the pairs CEL + CML and MOLD + GOLD each differ by one methyl group only, yet the Raman spectra are readily distinguishable.



(SEE panels B + C next slide)

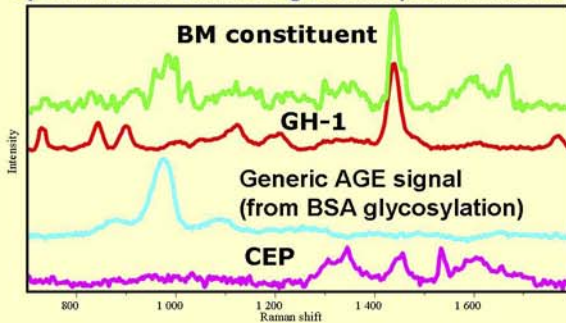
## Raman spectra of a range of AGE/ALE adducts that have been identified in human tissues



## RESULTS (II)

- Figure below shows one of the Raman signals recorded for BM tissue.
- Comparison with the spectral database shows the spectrum can be wholly accounted for by a linear combination of three adducts: **GH-1**, **CEP** and a **generic AGE signal** that was obtained by glycosylation of BSA. There were some shifts in band positions observed, which can generally be accounted for in terms of physical interactions with the matrix.

## Example of identification of biochemical constituents in BM by comparison of Raman spectrum with Raman signals in spectral database



## RESULTS (III)

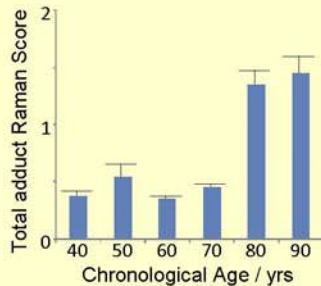
Using Raman as a probe enabled simultaneous tracking of 60 biochemical constituents including collagens, heme, cytochrome, fatty acid lipids, cholesterol, oxidised lipids and several AGE/ALEs:

**CML, CEL, CEP, GH-1, HHE, GO, DHP-lys, pentosidine**

The AGE/ALE signals all showed broadly the same trend and so were aggregated to produce the plot shown opposite

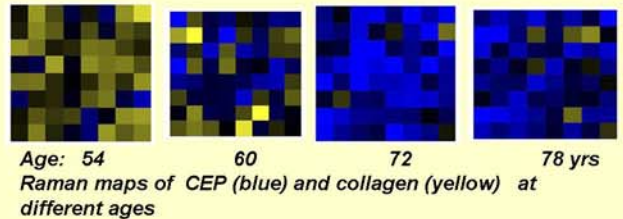
## Aggregated levels of AGE adducts as a function of age, probed by Raman

*the average level of AGE/ALE adduct vs chronological age*



## RESULTS (IV)

- The Raman signal can be mapped across a region of tissue to probe **spatial** distribution.



## CONCLUSIONS

- In order to understand the molecularly diverse range of AGEs/ALEs and their possible contribution to ageing mechanisms and AMD it is essential to employ a method capable of simultaneously measuring a significant number of these adducts *in the same cohort of donors*.  
*The present study clearly reveals the potential of Raman spectroscopy as a multiplexing tool in such an endeavour.*  
*The technique is also capable of probing the spatial distribution of constituents in ocular tissue at high resolution.*

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