Quantifying Therapy Effects on Diffusional Permeability in Human Bruch's Membrane Explants by MRI

J. M. Tyszka[1], C. Yu[1], I. K. Sugino[2], M. A. Zarbin[2], S. E. Fraser[1], and D. M. Schwartz[3]

[1]Division of Biology, California Institute of Technology, Pasadena, California, United States, [2]Institute of Ophthalmology and Visual Science, New Jersey Medical School, Newark, New Jersey, United States, [3]Department of Ophthalmology, University of California, San Francisco, San Francisco, California, United States

Introduction Age-related macular degeneration (AMD) is the leading cause of central vision loss in the US in patients older than 50. The vast majority of AMD cases are classified as "dry" AMD in which Bruch's membrane (a collagenous structure separating the retinal pigment epithelium from the subadjacent choriocapillaris) undergoes a number of changes including lipidization, protein crosslinking and protein deposition. Previous permeability studies of human Bruch's membrane have focused on age effects, but not on potential therapies (1,2). Our hypothesis is that reducing the lipid load in Bruch's membrane from donors over age 50 will increase membrane permeability, which in turn may delay or prevent progression to wet AMD. In this particular study we developed a non-invasive approach to quantifying membrane permeability using time-series MRI to follow ^2H_2O-^1H_2O equilibration across human Bruch's membrane explants and the effect of detergent treatment on the water transport kinetics.

Methods Human Bruch's membrane explants were obtained and prepared under institutionally approved protocols at the New Jersey Medical School. All MR data acquisition was performed using a 9.4T horizontal bore Bruker microimager, equipped with S116 gradients and a 35mm linear birdcage coil. Submacular sample pairs (right and left eye) were mounted within a customized Ussing's cell (Figure 1a) with a reference vial for signal normalization. The upper and lower chambers of each cell were filled with ^2H_2O PBS and ^1H_2O PBS respectively and sealed with Mylar tape. ^1H MRI of both cells was performed parallel to the cell axis using a conventional 2D double spin echo sequence (TR/TE = 1000/6ms, voxel size = 0.14x0.14x2mm, 3m12s per image, 40 repeats). Timecourse MRI was acquired pre- and post-treatment of the explant *in situ* with 1% Triton 100 detergent (30min at 37°C). The normalized mean ROI signal time-courses for all four chambers were analyzed by non-negative least squares (NNLS) curve fitting of an exponential basis set (Figure 1b). NNLS coefficient peaks were identified and analyzed statistically for the main effect of treatment and correlation with donor age.

Results and Discussion MR ROI time-courses for the upper and lower chamber demonstrated both mono- and multi-exponential behaviour. The change in permeability time constant did not correlate significantly with donor age. For sample pairs in which all decay behavior was monoexponential, the normalized difference in time constant between control and treated samples was -15% ($p = 0.045$, $n = 9$). For multiexponential decays, taking the amplitude weighted mean of the NNLS components, the normalized time constant difference was -11% ($p = 0.051$, $n = 15$). The main effect of treatment was therefore a consistent, but marginally significant (for $n = 9$) reduction in permeability time constant, equivalent to an increase in diffusional permeability due to detergent treatment.

Conclusions Magnetic resonance imaging allows quantification of water membrane permeability changes due to lipid extraction by detergent treatment. This study focuses on estimation of water diffusional permeability, but the apparatus and imaging methodology could be extended to dissolved species such as oxygen and paramagnetic contrast agents, allowing broad assessment of molecular transport of potential AMD therapies targeting lipid accumulation in Bruch's membrane.

References 1. Moore DJ, Clover GM. The effect of age on the macromolecular permeability of human Bruch's membrane. Invest Ophthalmol Vis Sci 2001;42(12):2970-2975. 2. Starita C, Hussain AA, Pagliarini S, Marshall J. Hydrodynamics of ageing Bruch's membrane: implications for macular disease. Experimental eye research 1996;62(5):565-572.

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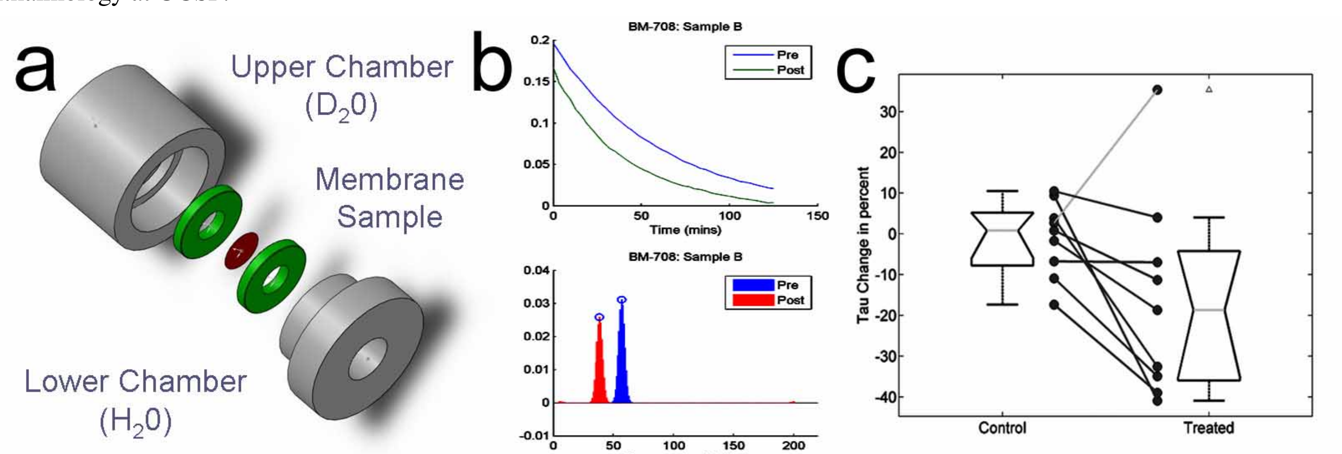

Figure 1: (a) MR-compatible, modified Ussing's chamber schematic. (b) Example NNLS exponential decay analysis of the signal difference between the chambers pre and post-detergent treatment of the sample. (c) Paired analysis of control and treated membrane permeability indices for mono-exponential decay samples demonstrating a consistent decrease in the equilibration time-constant following detergent treatment.