Quantifying Solute Diffusivity in Human Osteoarthritic Cartilage via Correlation Spectroscopy

Alison D. Wright[1], Brian T. Graham[2], Christopher Price[1,2]
[1]Biomedical Engineering, [2]Mechanical Engineering, University of Delaware

Introduction: Osteoarthritis (OA) is a disease characterized by progressive articular cartilage degeneration. In the early stages of OA, symptoms often go undetected, as changes in the cartilage occur at the microscale level (i.e. sub-clinically). Such early changes include alterations to the tissue's composition and microstructure.[1] Changes in the microstructure of the matrix during early OA should lead to changes in molecular diffusivity in cartilage. A diagnostic technique sensitive enough to detect changes in the diffusive environment of cartilage may allow for earlier disease identification (i.e. diagnosis) and implementation of intervention strategies. Two techniques capable of quantifying molecular self-diffusivity in hydrated tissues are fluorescence correlation spectroscopy (FCS) and raster image correlation spectroscopy (RICS); however, neither have been investigated in osteoarthritic tissues.[2-4] The goals of this study were to 1) demonstrate the ability of FCS and RICS to assess solute diffusivity in human cartilage explants, 2) determine if OA progression drives identifiable differences in diffusivity, and 3) determine if these changes in diffusivity correlate with changes in mechanical and compositional properties.

Materials and Methods: Human cartilage retrieved from the femoral condyles of five patients undergoing total knee arthroplasty were scored per International Cartilage Repair Society (ICRS) guidelines. Full thickness cartilage plugs (3 mm ø) were taken from areas of ICRS scores 0, 1, and 2 **(Fig. 1A)**. FCS and RICS were used to determine the diffusion coefficient of a small (fluorescein 332Da) and mid-size (Texas Red-conjugated 3kDa dextran) solute within each plug. Creep relaxation indentation testing was used to determine compressive modulus (E_{y-}), tensile modulus (E_{y+}), the unstrained initial permeability (k_0), and the strain dependent permeability-modifying coefficient (m). Dimethylmethylene blue (DMMB) and hydroxyproline assays were used to determine GAG and hydroxyproline content, respectively.

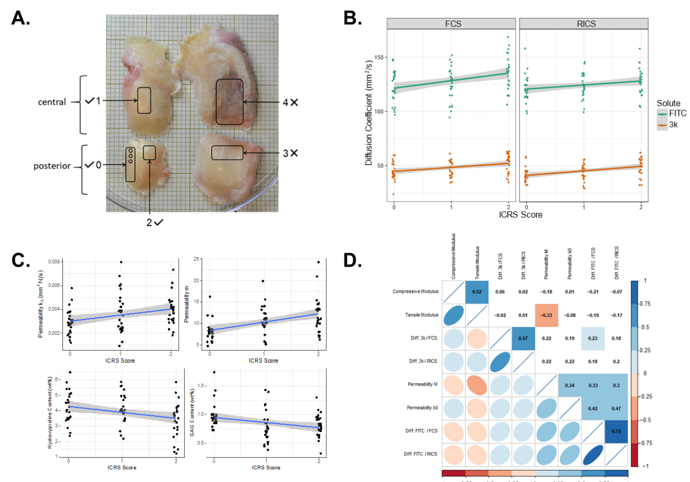

Figure 1. A) Femoral condyle cuts from a TKA patient exhibiting regions of each ICRS score. B) Diffusion coefficient for each solute measured by FCS and RICS significantly increased with increasing ICRS score ($p<0.05$). C) Significant increases in permeability (k0 and m) and significant increases in GAG and hydroxyproline content were observed with increasing ICRS score ($p<0.05$). D) Pearson correlation coefficient matrix of all experimental parameters.

Results and Discussion: The diffusivity of each solute, as measured by FCS and RICS, were found to increase significantly with increasing ICRS score **(Fig. 1B)**. Both permeability coefficients, k0 and m, also increased with increasing ICRS score **(Fig. 1C)**. Non-significant decreases in compressive and tensile moduli accompanied increases in ICRS score. GAG and hydroxyproline content significantly decreased with increasing ICRS score. These relationships, between diffusive, mechanical, and compositional parameters and ICRS scores, align with predictions regarding the effect of OA and ECM deterioration on individual cartilage matrix properties. Further examination of the relationships between each sample's diffusion, mechanical, and compositional properties was performed using Pearson correlation coefficients **(Fig. 1D)**. Most notably, significant positive correlations between fluorescein diffusivities obtained via FCS and RICS and permeability were observed.

Conclusions: This study demonstrated the ability of FCS and RICS to detect changes in solute diffusivity between healthy and early stage OA cartilage, suggesting that these techniques may be used as a diagnostic tool for earlier identification of OA. Future studies will investigate the sensitivity of FCS and RICS to detect the earliest changes in ECM properties associated with cartilage injury and OA initiation, and will look to marry FCS and RICS-based diffusion assessment methodologies with confocal arthroscopy techniques to assess the ability of correlation spectroscopy to assess cartilage health in vivo.

References: [1] Setton LA, *Osteoarthr. Cartil.* 1999, 7(1):2-14. [2] Elson EL, *Biopolymers* 1974, 13:1-27. [3] Magde D, *Biopolymers* 1974, 13(1):29-61. [4] Digman MA, *Biophys. J.* 2005, 88(5):L33-L36.