Molecular Diffusion in Articular Cartilage and Tissue Engineered Cartilage Constructs

Introduction: Articular cartilage is an avascular tissue, suggesting that the transport of nutrients and metabolites through the tissue occurs primarily by diffusion or convection. Alterations in the rates of molecular diffusion, which may occur with aging or disease, therefore have the potential to influence chondrocyte metabolism by limiting the transport of nutrients or signaling molecules. Similarly, the transport of such molecules in tissue-engineered cartilage constructs is also controlled by diffusion. Molecular diffusion is affected by the structure and composition of the tissue, as well as by the size of the diffusing molecule. For example, removal of proteoglycans by enzymatic degradation alters the diffusion coefficient in a manner that depends upon the size of the diffusing molecule [1]. Removal of the cartilage surface zone decreases the diffusion coefficients of smaller molecules (10 kDa dextran), but increases the diffusion coefficient of a larger 70 kDa dextran [2]. Because the composition of cartilage changes with depth from the tissue surface, we hypothesized that the diffusivity in cartilage varies with depth. Furthermore, we hypothesized that the diffusion coefficients of tissue-engineered cartilage constructs will change with time in culture, as the cells produce matrix and the scaffold degrades. An important advance of our study was the application of fluorescence recovery after photobleaching (FRAP), a technique that allows measurement of site-specific diffusion coefficients in cartilage and in tissue-engineered cartilage constructs.

Materials and Methods: Native Cartilage Specimens: Full-depth explants (5mm diam. x 2mm thick) of articular cartilage were harvested from the femoral condyles of 2 year-old female pigs. Samples were incubated in fluorescein conjugated dextrans (3, 40, 70, and 500 kDa), suspended in phosphate buffered saline. FRAP experiments were performed in the surface, middle, and deep zone of each specimen. Diffusion coefficients were calculated by measuring the half recovery time and size of a circular bleached region in the tissue (radius ~37 µm) [3]. Tissue Engineered Constructs: Scaffold disks (6-8 mm diameter) made of 2% w/v low melting point agarose, 2% w/v low viscosity sodium alginate, Surgifoam porous gelatin sponges (J&J), and fibrin from (Tissucel VH two-component fibrin sealant, Baxter) were seeded with human adipose derived adult stromal cells at 10^7 cells/ml. Scaffolds were cultured in control media (DMEM-b, 10% FBS, 1% penicillin/streptomycin) or chondrogenic media (control media plus 1x insulin-transferrin-selenium supplement, 37.5 µg/ml ascorbate, 10 ng/ml TGF-β1, and 100nM dexamethasone) at 37°C and 5% CO₂ for 1 hour (day 0) or 7 days [4]. Diffusion coefficients were measured as above using the 70kDa dextran. Statistical analysis was performed using ANOVA with an LSD post hoc test (α=0.05).

Results: In native articular cartilage, diffusion coefficients decreased significantly with increasing molecule size when averaged across all zones (p<0.00001, n=26). For the 3 kDa dextran, the diffusion coefficient in the surface zone was significantly greater than that of the middle and deep zones (p<0.01, n=15 per group) (Figure 1). In contrast, the diffusion coefficients of the 40 and 70 kDa dextrans were significantly lower in the surface zone than in the middle and deep zones (40 kDa: p<0.05, n=7 per group; 70 kDa: p<0.0005, n=13 per group). The 500 kDa dextran showed the same trend as the 3 kDa dextran with the diffusion coefficient in the surface zone being significantly greater than the diffusion coefficients in the middle and deep zones (p<0.001, n=14 per group).

All tissue engineered constructs had diffusivities that were at least double those of native cartilage (Figure 2). The diffusion coefficients of all constructs in chondrogenic conditions decreased significantly from day 0 to day 7 (p<0.05, n=5 per group). Chondrogenic treatment significantly decreased the diffusion coefficient, as compared to control, in Surgifoam after 7 days in culture (p<0.05, n=5 per group). When all times and conditions are pooled, the mean diffusion coefficient of fibrin was significantly lower than all other scaffolds (p<0.0007), the mean diffusion coefficients of alginate and agarose were not significantly different (p=0.3), and the mean diffusion coefficient of Surgifoam was significantly greater than all other scaffolds (p<0.001).

Discussion: The diffusion coefficients measured with FRAP in cartilage were similar to those measured previously by Torzilli et al. [2] and Quinn et al. [4] using radiotracer movement or fluorescence desorption. Significant differences were observed in diffusivity between the surface zone and the middle and deep zones of native cartilage. However, no differences in were observed in diffusivity between the middle and deep zones. Presumably, the zonal differences in diffusion coefficients are due to the molecular interactions with extracellular matrix molecules that act as barriers to diffusion. The structure and concentration of these molecules (e.g., collagen and proteoglycans) in cartilage change with zone [5], and therefore the primary barriers to diffusion in each zone may vary with the size of the molecule.

The observed decrease in diffusivity with time in tissue engineered cartilage constructs is likely to reflect the accumulation of newly synthesized extracellular matrix components. Furthermore, fibrin and Surgifoam scaffolds contracted with time in culture, potentially decreasing the effective pore size and therefore the diffusivity of the matrix. On the other hand, acellular scaffolds exhibited a slight increase in diffusivity over the test period, which would be expected from scaffold degradation alone. The net decrease of diffusivity in the cell-seeded scaffolds implies that the cells are exerting a strong effect on the diffusion coefficients in the scaffolds through the production of new matrix and the active transport of molecules and ions.

In summary, our findings indicate that variations in the structure and composition of native articular cartilage and tissue engineered cartilage constructs significantly affect the rates of molecular diffusion. An understanding of the diffusion properties of native and engineered cartilage will be important for our understanding of chondrocyte physiology and pathophysiology and will improve the functional tissue engineering of articular cartilage [6].

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Figure 1. Diffusion coefficients of different sized dextrans vary significantly between zones in articular cartilage. Bars are mean plus standard error. Asterisks indicate significant difference between the marked bar and the other two zones for each molecule (p<0.05, ANOVA).

Figure 2. Diffusion coefficients of 70kDa dextran in tissue engineered cartilage constructs vary between scaffold materials, with time in culture, and with control or chondrogenic culture conditions. Asterisks indicate significant difference between day 0 and day 7 (p<0.05, ANOVA). # indicates significant difference between control and chondrogenic culture conditions (p<0.05, ANOVA).

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