

## Enzymatic treatment to improve the permeability of cartilage

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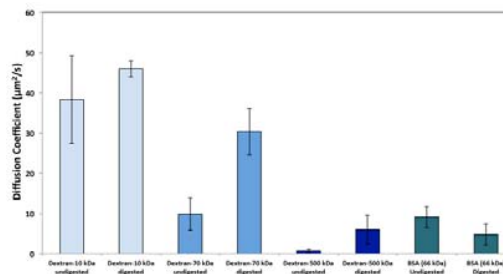
**Statement of Purpose:** Cartilage is an avascular tissue and chondrocytes are surrounded in a dense extracellular matrix (ECM), in which the transport of the nutritive occurs through continuous diffusion instead of through the vasculature. The metabolic activity of cartilage tissue is nourished solely by diffusion and the dense ECM hinders the ability for chondrocytes to proliferate and migrate. Hence the cartilage defects show a very limited self-repair capacity. Techniques used clinically for cartilage repair include debridement, bone marrow stimulation, mosaicplasty and autologous chondrocyte implantation (ACI). However, currently none can predictably restore articular surface, the repair tissue eventually degenerates to fibrocartilage and the symptoms return.

Several studies have shown an improved capacity for cartilage repair by enzyme treatment, possibly by increasing the cell density at cartilage wound edges. Diffusion in ECM of cartilage plays a central role in the physiobiological nature of chondrocytes, but very few studies have examined the diffusion behaviors of cartilage after enzymatic treatment. The aim of the present study was to investigate the diffusive behavior of different molecules in enzymatically treated cartilage and the structural changes in ECM after enzymatic treatment.

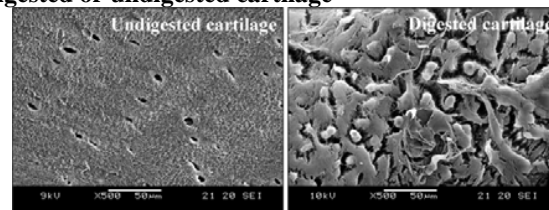
**Methods:** Cartilage samples were harvested from the porcine femoral condyles. Full thickness harvested tissue was cut out into 5 mm by 1 mm by the thickness of cartilage. Then samples were partially digested in a collagenase and protease blend (Liberase) at 37°C for 20 minutes, followed by immersing in FITC-dextran or FITC-BSA solution at 4°C for 2 days. Fluorescence recovery after photobleaching (FRAP) was performed to investigate the behavior of the fluorophores in cartilage and their diffusion coefficients were calculated. To examine the structural changes in ECM after enzymatic treatment, SEM was used to observe the ECM structure. Samples without enzymatic treatment were used as control group.

**Results:** As shown in Fig. 1, the diffusion coefficients were inversely proportional to dextran size for both partial digested and undigested cartilage; 10 kDa FITC-dextran showed the highest diffusion coefficient, followed by the 70 and the 500 kDa FITC-dextran. The diffusion coefficients were correlated to the inverse of the published hydrodynamic radii of the dextrans as predicted by the Stokes–Einstein relationship. The enzymatic treatment resulted in a significant increase in the diffusion of the dextran molecules. Compared to controls, the diffusion coefficient of 10 kDa FITC-dextran increased by 1.23 times in the cartilage treated with enzyme, while 70 kDa and 500 kDa FITC-dextran increased by 3.56 and 10.87 times respectively. However, the diffusion coefficient of FITC-BSA in digested cartilage was lower than in undigested cartilage.

Changes in the ECM microstructure were compared under SEM. As shown in Figure 2, the surface of undigested tissue showed a fibrous collagen network with lacunae and no chondrocyte were present on the surface. In contrast, the collagen network was disrupted and the embedded chondrocytes were exposed after enzymatic treatment.



**Figure 1. Diffusion coefficients of dextran and BSA in digested or undigested cartilage**



**Figure 2. SEM observation of cartilage surface**

**Conclusions:** FRAP experiments showed that the permeability of cartilage was enhanced after enzymatic treatment. The increase in the diffusion coefficient may result from the disruption of the ECM by enzymatic digestion. The size of dextran examined in this study was selected to mimic certain physiologically relevant molecules. The large 500 kDa dextran was similar in size to fibronectin or cartilage oligomeric protein which have molecular weights of 550 or 500 kDa. The 70 kDa dextran was similar in size to some of the smaller matrix molecules such as biglycan and decorin, and the 10 kDa dextran was similar in size to important growth factors such as BMP-7, BMP-3, IGF and EGF. Interestingly, diffusion coefficients of FITC-BSA in partially digested cartilage were lower than in undigested cartilage. It is possible that an aggressive and short-term partial digestion that induces disruption of the cartilage ECM may change the natural space of the ECM to a much more intricate environment with exudates of digested ECM constituents hindering BSA diffusion and it may even be possible that the partial digestion exposes non-specific binding sites on the collagen with BSA. Moreover, this finding may also imply that the enzymatic treatment preserved most of the structural ECM in cartilage.

### References:

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