

Use of Novel Chitosan Hydrogel Cross-Linkers for Photo/Chemical Bonding of Osteochondral Transplants

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Summary:

This study introduces a new tissue bonding technique that has the potential to circumvent complications related to osteochondral transplants by successfully preventing fluid influx at the graft-host interface.

Abstract:

Introduction:

Autologous osteochondral grafts are presently the most successful surgical approach for repair of articular cartilage defects. However, there are several factors which threaten the durability of the treatment and trigger the occurrence of premature osteoarthritis. Repair of the graft-host interface occurs with the formation of fibrocartilage, which possesses inferior mechanical properties compared to normal hyaline cartilage.¹ Moreover, the discontinuity at the transplant-host interface allows synovial fluid influx from the joint during weight bearing. This invasion of fluid is believed to be the cause of reoccurring subchondral cysts seen in patients following osteochondral transplantation.² Therefore, elimination of this healing limitation is essential to create an environment that promotes the migration of chondrocytes, reconstruction of their extracellular matrix and efficient transfer of compressive loads. It has been previously shown that the use of cross-linkers, combined with a pretreatment that removes proteoglycans (via enzymatic degradation), enables the bonding of articular cartilage.³ This study explores the use of chondroitinase-ABC (Ch-ABC) and three different polymer/cross-linker combinations: chitosan and genipin (Chi-GP); chitosan and rose bengal (Chi-RB); and chitosan, rose bengal and genipin (Chi-RB-GP). Each combination was evaluated on its efficiency to improve the cartilage-to-cartilage interface, based on adhesion strength and permeability.

Methods:

14.5 mm biopsy punches were used to remove articular cartilage plugs from the trochlea and femoral condyles of immature bovine joints. These plugs were sliced into 2 mm thick discs and a 5 mm defect was created using a biopsy punch, establishing an annulus and inner core. As a pretreatment, Ch-ABC was brushed onto the inner surface of the annulus and outer surface of the core, and incubated for 15 min. A mixture of chitosan and a cross-linker was then introduced to the surfaces of the annulus-core interface. Each polymer/cross-linker combination was tested with and without the use of Ch-ABC. The specimens treated with Chi-GP were incubated for 15 min at room temperature to allow diffusion of the mixture within the tissue and efficient initiation of the gelation process. Chi-RB and Chi-RB-GP samples were first incubated for 15 min, and then exposed to visible light for another 15 min period. For the control group, the cores were inserted into their respective annuli without any treatment and incubated in PBS. Post tissue bonding, push-out tests were performed to determine the adhesion strength at the interface. The bonded cartilage explants were placed into a stainless steel loading rig and a force was applied at a constant speed until the bond was broken. The adhesion strength was defined as the maximum force required to break the bond divided by the interfacial surface area. The permeability of the specimens in their intact, defect (without any treatments) and treated states was also assessed. The cartilage explants were placed in a pressurized chamber and the amount of

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fluid which passed through the cartilage over a 15 min period was recorded as the fluid velocity. The permeability was then calculated using Darcy's law. One- and two-way ANOVA with Tukey post-tests were respectively used to statistically analyze the adhesion strengths and fluid velocities for each condition and treatment.

Results:

Cartilage explants pretreated with Ch-ABC produced significantly higher adhesion strengths than specimens treated without Ch-ABC, thus only Ch-ABC data is presented. When compared to the control, all of the treated explants produced significantly higher adhesion strengths ($n=5$, $p<0.05$, Figure 1). The Chi-RB-GP treatment resulted in significantly superior adhesion strength in comparison to the other two treatments ($n=5$, $p<0.05$, Figure 1). For permeability testing, creating a defect increased fluid velocity, while treating the defect restored fluid velocity to that of the intact state. Indeed, there was no statistically significant differences in fluid velocity found between the intact and treated specimens in all groups ($n=9$, $p<0.05$, Figure 2). Statistical significance between the defect and treated fluid velocity values was only observed in the Chi-GP treatment ($n=9$, $p<0.05$, Figure 2).

Discussion:

Enzymatic degradation with Ch-ABC exposed collagen fibers to increase the number of available cross-linking sites at the donor-recipient interface. This had a positive effect on the coherence between the two cartilage surfaces. Chitosan used in combination with cross-linking reagents provided strong interfacial mechanical properties, while the Chi-GP duo provided superior restoration of the permeability at the area of cartilage divergence. Future studies will be conducted to determine chondrocyte viability in treated and untreated explants in vitro. Cytotoxicity tests will yield further determining factors for future preclinical investigations involving in vivo testing of treatments in an animal model.

Significance:

This study introduces a new tissue bonding technique that has the potential to circumvent complications related to osteochondral transplants. The proposed treatments provided a continuous interfacial region which successfully prevented fluid influx at the graft-host interface and allowed load transfer. Consequently, this approach could potentially be used in a clinical setting to improve articular cartilage healing following osteochondral transplantation.

References:

1. Horas, U, et al. *J Bone Joint Surg Am*, 85-A(2):185-192 (2003).
2. Valderrabano, V., et al. *Am J Sports Med*, 1:105S-111S (2009).
3. Sitterle, V. B., et al. *Osteoarthritis and Cartilage*, 17:1656 (2009).