NATIVE/ENGINEERED CARTILAGE ADHESION VARIES WITH SCAFFOLD MATERIAL AND DOES NOT CORRELATE TO GROSS BIOCHEMICAL CONTENT

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INTRODUCTION While many studies have examined the in vitro behavior of tissue engineered cartilage (1), few have characterized interactions between the construct and the surrounding native tissue. Successful development of tissue engineered cartilage will require both proper matrix synthesis and integration into the surrounding tissue (2,3). Many different tissue engineering strategies have been developed, and some studies have examined the ability to integrate into the surrounding cartilage (4,5). However, the detailed relationship between construct maturation and integration into the surrounding tissues is largely unexplored. This study compared the behavior of four different engineered cartilages in a hybrid culture system, specifically investigating the associations between construct matrix composition and interfacial strength.

METHODS Full-thickness cores of articular cartilage (8mm diam) were isolated from the femoral condyles and patello-femoral grooves of immature cattle, and chondrocytes were isolated from the remaining cartilage via collagenase digestion. The cartilage cores were trimmed to 3mm thickness, and circular holes (4mm diam) were cut to form annuli of tissue with 40 µl defects (Fig. 1), and implanted into defects using one of three scaffolds: PGA, fibrin, or agarose (n=6). PGA discs were seeded with chondrocytes (256 cells/ml) and cultured for either 5 days (immature; "PGA(i)") or 5 weeks (mature; "PGA(m)"), after which 4mm diameter cores were punched from the discs and press-fit into fresh defects. Fibrin (50 mg/ml) and agarose (2% w/v) gels, with 100 cells/ml were cast directly into defects and polymerized in situ. Constructs were cultured for 20 or 40 days then analyzed mechanically (push-out failure test, Fig. 2) and biochemically (DNA, sulfated glycosaminoglycan, and hydroxyproline content). As a measurement of interfacial strength, the failure stress (max force/interface area) and energy to failure (area under the force-displacement curve) were calculated. In order to compare the interfacial strength with tissue-tissue adhesion, hybrids were cultured wherein the tissue core was punched out and then replaced into the defect. Data were analyzed using ANOVA and Tukey’s test for post-hoc analyses (significance at p<0.05) and the Pearson’s product moment correlation.

RESULTS Day 20 After 20 days in culture, PGA(m) constructs exhibited the greatest failure stress, while fibrin constructs exhibited the greatest energy to failure (Fig. 3). Tissue-tissue hybrids exhibited significantly higher failure stress (28.6±8.7 kPa) and energy to failure (0.087±0.01 mJ). PGA(m) constructs contained the most hydroxyproline, while agarose constructs contained the most hydroxyproline. PGA(i) treatments (Fig. 4).

Day 40 After a further 20 days in culture, fibrin constructs exhibited both the greatest failure stress and the greatest energy to failure. Failure stress increased from day 20 to 40 for fibrin gels, was unchanged for agarose gels and PGA(i), and decreased for PGA(m). Failure stress and energy to failure were unchanged for tissue-tissue hybrids (33.3±7.0 kPa and 0.22±0.06 mJ, respectively). PGA(m) constructs still contained the most hydroxyproline, and agarose contained the most sGAG. There was a significant difference in DNA content between the different scaffolds, with agarose containing the most and PGA(i) the least DNA. Both fibrin and agarose showed accumulation of sGAG and DNA from day 20 to 40, while there were no significant changes in either PGA condition. Hydroxyproline content was unchanged in fibrin and PGA(i), and had decreased in both agarose and PGA(m). Across scaffold conditions, neither failure stress nor energy correlated with DNA, sGAG, or hydroxyproline content at either time point.

DISCUSSION Adhesion and integration between the native and repair tissues will be an important determinant of long-term success for tissue engineered repair of articular cartilage. In this study, we investigated the adhesion of four different engineered cartilages in an in vitro defect repair model. It is interesting to note that none of the scaffold systems developed adhesions as strong as that between two pieces of living explant tissue. There were marked differences between the scaffolds, but neither failure stress nor energy to failure correlated to any measure of total biochemical content. Thus we conclude that measures of gross composition (DNA, sGAG, hydroxyproline) may not be adequate predictors of interface mechanical integrity. We have not yet studied the detailed mechanisms involved in adhesion, and it remains to be seen whether other biochemical factors, such as the degree of crosslinking between collagen fibers, may be better predictors of adhesive strength. Inhibition of crosslink formation has been shown to result in a weaker bond between pieces of explant tissue (5), and may have similar effects on engineered cartilages. Identification of reliable predictors of adhesive strength may be extremely useful for evaluating different tissue engineering strategies before performing animal and clinical studies.

ACKNOWLEDGEMENTS This work was supported by the ERC Program of the NSF under Award Number EEC-9731643, by the NIH under the Cellular Engineering Training Program, grant number GM08433, and by a grant from the Medtronic Foundation.


48th Annual Meeting of the Orthopaedic Research Society
Poster No: 0479