INTRODUCTION: Articular chondrocyte transplantation is one of the few surgical techniques utilized for articular cartilage repair [1]. The ability of new cartilage to adhere to native cartilage is a crucial issue to consider if such an approach is to be successful [2]. In our previous work, we have demonstrated that newly formed matrix from isolated chondrocytes is capable of bonding pieces of articular cartilage matrix together [3] with biomechanical integrity [4,5] for up to 6 weeks in vivo. The objective of this study was to investigate the bonding characteristics of newly formed cartilage matrix over a period of 8 months in nude mice and to analyze the behavior of a cartilage/chondrocyte construct in extended implantation.

MATERIALS AND METHODS: Articular cartilage discs (8 mm x 1 mm) were harvested from pig joints and devitalized by multiple (5) freeze-thaw cycles. Chondrocytes were isolated from cartilage from animals of the same species by collagenase digestion. The devitalized cartilage discs were incubated in the presence (cellular density of 0.2 million/ml) or absence of chondrocytes in suspension culture in Ham F12 media with 10% FBS, 50 mg/l ascorbate, and antibiotics for 21 days in order to allow chondrocytes to adhere to their surface. After in vitro culture, pairs of cartilage discs were held in apposition in fibrin glue and implanted in nude mice in subcutaneous pockets for up to 8 months. Two experimental samples (with cells) and two controls (without cells) were implanted in each mouse. Groups of 10 mice were sacrificed at 1, 2, 4, and 8 months after implantation. Upon harvest, samples were either frozen at -80°C for mechanical testing or were fixed in formalin, sectioned, and stained with Safranin-O for morphologic evaluation. The mechanical integrity of bonded cartilage pieces was evaluated by tensile testing on a Dynastat mechanical spectrometer as described previously [4]. Chondrocyte/matrix constructs were attached to plexiglass rods using cyanoacrylate glue, and rods were mounted in the Dynastat. Tensile displacements were applied at a rate of 10 μm/s to failure and the resultant stress-strain curves. Sample displacements and loads were normalized to strain and stress by sample geometry and ultimate tensile strength, failure strain, strain energy density, and tensile modulus were calculated from the resultant stress-strain curves.

RESULTS: In control samples that were wrapped in only fibrin glue without cells, no matrix formation was observed at 1 month (fig. 1A, 100x) or any later times. Areas of calcification have been noted in the bulk of the cartilage discs especially at the longer times in the control samples. Examination of histology of experimental specimens showed that newly formed cartilage matrix adhered to devitalized matrix leading to a complete fusion of the two matrix discs (fig. 1B, 100x). Degradation of the cartilage matrix of the scaffold discs were also noted in the control specimens 4 and 8 months (fig. 1C, 100x) after implantation. This degradation was not noted in experimental samples, which showed the presence of new cartilage bonding devitalized pieces out to 8 months (fig. 1D, 100x).

A two way analysis of variance (ANOVA) with a post-hoc Tukey test was performed on mechanical property data. Ultimate tensile strength (fig. 2A), failure strain (fig. 2B), and strain energy density (fig. 2C) increased with time for the experimental group with no increases in time for any properties of control samples. Tensile modulus (fig. 2D) did not increase significantly with time. All mechanical properties were significantly higher in experimental than in control samples at 4 and 8 months, with ultimate tensile strength significantly greater by 2 months as well.

DISCUSSION: This study investigated the biomechanical properties and the morphologic characteristics of cartilage matrix formed from isolated chondrocytes in a long term in vivo study. Other studies have examined the ability of live cartilage explants to bond in vitro [6], as well as the capability of isolated articular chondrocytes to achieve bonding between devitalized articular cartilage matrices with biomechanical integrity in vivo in 6 weeks [3,4]. Results from the current study indicated an ultimate tensile strength of ~90 kPa after 8 months, which was approximately 20% higher than that seen at 6 weeks. By 8 months, failure strain and failure energy were both 4-5 times higher than that reported at 6 weeks [4] indicating a continued remodeling of the newly formed matrix. Modulus values were similar to those reported previously. No significant differences were noted between 4 and 8 months, suggesting a stabilization of the system at 4 months. The degradation of control samples that was not seen in experimental samples suggests that chondrocyte seeding provides a "protective" effect on the scaffold matrix, although the mechanism of this phenomenon is not known. Future studies will also analyze the influence of pre-implantation culture conditions such as the addition of growth factors, cell concentration, and seeding duration on the adhesion properties.


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