INTRODUCTION: Chondrocyte-based methods of articular cartilage repair may depend on the adhesion of cells to the surrounding host cartilage. In preparation for transplantation, chondrocytes are typically released from monolayer culture with trypsin [1]. In addition, treatment of host cartilage with chondroitinase ABC (ChABC) has been advocated to enhance cell adhesion [5]. The molecular basis of adhesion of chondrocytes to cartilage under such repair conditions is unknown, although various cell-surface receptors including β1-integrins [8], anchorin CII [9], and CD44 [7] are known to mediate chondrocyte-cartilage interactions. The objective of this study was to quantify the contribution of β1-integrins, anchorin CII, and CD44 to the strength of chondrocyte adhesion to cartilage in vitro.

METHODS: Cell Culture: Chondrocytes were isolated from femoral condyle articular cartilage of mature bovines by sequential digestion with pronase and collagenase, plated at 200,000 cells/cm², and cultured for 6-10 days [8] in DME+10% FBS. Cells were stained overnight with 10 μM CMFDA, a fluorescent cytoplasmic dye. Prior to use, cells were trypsinized, rinsed twice in DME+10% FBS, re-suspended, and allowed to recover before use.

Explant Preparation: Fifty μm thick bovine cartilage explants from the patellofemoral groove were cryostat sectioned parallel to the articular surface and stored at -80°C. Before use, sections were thawed in PBS, treated for 5 minutes with 1 μM clinical grade ChABC (Seikagaku), and rinsed 6 times in 1 ml medium over 6 minutes. The amount of GAG digested from and remaining in the tissue was quantified by OD232 [11] and dimethylmethylene blue [3], respectively.

Adhesion Assay: A parallel plate shear flow chamber (Fig. 1) was modified to include a cartilage explant as a substrate for cell attachment. Images, captured before applying shear stress and 1 minute after, were analyzed to assess cell detachment.

Statistical Analysis: Non-Gaussian data were transformed by a modified arcsine function [12], and comparisons at each shear stress level were made by ANOVA and Dunnett’s post-hoc test. Data are means±SD.

RESULTS: Treatment with ChABC to enhance cell adhesion [5] removed 46±3% (5±3 μg) of the total GAG (166±30 μg) from the tissue. This was estimated to correspond to a digestion depth of 1±1 μm.

There was a significant effect of treatment with blocking reagents at applied shear stresses of 6, 28, and 70 Pa (each, ANOVA p<0.001, Fig. 2). At each of these shear levels chondrocyte detachment was significantly increased for samples treated with anti-β1 mAb (p=0.01 or 0.001). The adhesive strength (defined as 50% detachment) of control chondrocytes after 20 min seeding time was 54±20 Pa. Pre-treatment of cells with anti-β1 mAb resulted in a decrease in adhesive strength to 7±4 Pa. Additional control experiments confirmed that the low (0.001%) concentration of NaN3 present in the 4B4 antibody solution had no detectable effect on cell adhesion. Cell detachment was similar (p=0.3-0.9) with (n=4) and without (n=4) 0.001% NaN3 for each level of shear.

DISCUSSION: In cartilage repair procedures involving transplantation of chondrocytes, attachment of cells to the apposing host articular cartilage surface may be critical for graft-host integration. Delineation of the mechanisms of adhesion in terms of specific chondrocyte receptors and cartilage tissue ligands may allow cell manipulations or matrix treatments to enhance adhesion. In the ex vivo system studied here, the apparent involvement of β1-integrins, but not CD44 or anchorin CII, may reflect selective receptor degradation following trypsin treatment [7], incomplete receptor blocking (low affinity of the HAb4mab, receptor occupancy [9] or steric factors. Quantitative measures of chondrocyte adhesion allow direct comparisons of the effectiveness of potential treatments. Such measures also allow analysis of the possible induction of cell detachment in vivo by mechanical stress during and following transplantation.


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