ADHESIVE FORCE OF CHONDROCYTES TO CARTILAGE: A MICROPIPETTE STUDY OF THE EFFECTS OF CHONDROITINASE ABC

INTRODUCTION: Articular cartilage has a limited capacity for repair after injury. Attempts recently have been made to develop new methods to enhance the repair of full thickness defects. Some procedures have utilized cells implanted in agarose gel and cultured in combination with scaffolding materials. Even with these procedures, defects of articular cartilage fail to heal, possibly due to the inability of repair cells to adhere to cartilage matrix surface [1]. It has been hypothesized that cell adhesion can be inhibited by proteoglycans in articular cartilage [2]. Recently, it has been reported that repair cell adhesion is enhanced by enzymatic degradation of proteoglycans on the surface of partial-thickness cartilage defects in vivo, using 1 Unit/ml Chondroitinase ABC (ChABC, from Sigma) for 5 min [1,3]. Previous studies indicate that chondrocytes adhere to cartilage explants in vitro [4,5]. The purposes of the present study were to use an in vitro approach (1) to make quantitative measurements of the adhesiveness of individual chondrocytes to cartilage using the micropipette technique, (2) to determine if treatment of cartilage with Chondroitinase ABC at various concentrations and durations affects chondrocyte adhesiveness, and (3) to determine if chondrocytes adhere differently to superficial, mid, and deep regions of cartilage.

MATERIALS AND METHODS: Cell Isolation. Chondrocytes were isolated from full thickness articular cartilage of the femoral condyles of mature bovines by sequential enzymatic digestion with hyaluronidase and chondroitinase ABC at various concentrations and durations. Enzymatic digestion of cartilage. Using ChABC at three concentrations and two treatment times, the effective combinations to enhance chondrocyte adhesion were 0.5 unit/ml for 15 minutes and 1.0 unit/ml for 5 or 15 minutes. (3) Using ChABC digestion of cartilage, the adhesion force of transplanted chondrocytes increased with ChABC treatment times, the effective combinations to enhance chondrocyte adhesion were 0.5 unit/ml for 15 minutes and 1.0 unit/ml for 5 or 15 minutes.

RESULTS: The chondrocyte adhesion force (Fig. 1) showed a dependence on seeding time (p<0.001), ChABC treatment (p<0.001), but not region of tissue (i.e., superficial, mid, deep) to which the cells were attached (p=0.39). For normal cartilage, the adhesion force increased from 1.29±0.24 to 5.29±0.25 mdyn. Treatment with ChABC at a relatively high concentration and/or long treatment time (1.0 Unit/ml for 5 min, or 0.5 or 1.0 Unit/ml for 15 min) led to a significant (p<0.001) increase in adhesion force, whereas relatively low concentration / treatment time (0.25 Unit/ml for 15 min, 0.5 Unit/ml for 5 min) had no significant effect (p=0.87, 0.89). The increase in adhesion due to ChABC treatment appeared most marked for the short (15-30 min) seeding duration.

DISCUSSION: The major findings of our study are as follows. (1) The adhesion force of the transplanted chondrocytes to articular cartilage cross sectional surface through the depth of the tissue increased with seeding time. (2) The adhesion force of transplanted chondrocytes increased with ChABC digestion of cartilage. (3) Using ChABC at three concentrations and two treatment times, the effective combinations to enhance chondrocyte adhesion were 0.5 unit/ml for 15 minutes and 1.0 unit/ml for 5 or 15 minutes. The results provide direct biomechanical evidence that enzymatic treatment of a cartilage surface can enhance chondrocyte adhesion. The use of a sufficient enzyme concentration and treatment duration was required for a detectable effect, and the effect was most marked at early seeding times (within 30 min). The method of preparation of transplanted chondrocytes and tissue surfaces in a repair situation may affect the adhesion of cells as well as matrix deposition, and thus be critical for achieving successful integration between implanted cells and apposing host articular cartilage. The increase in adhesion force of individual chondrocytes with seeding duration is consistent with previous studies [5] in which the adhesive strength of large populations of chondrocytes to cartilage was determined after 5-40 min of seeding by application of fluid-induced shear. The micropipette aspiration method allowed the additional assessment of individual cell adhesion to different regions of cartilage (i.e., superficial, mid, and deep layers). The lack of a regional effect remains to be explained, in view of the structural and compositional matrix variation with depth from the articular surface.


ACKNOWLEDGMENT: This research was supported by NIH AR14918 and AR44658, NSF BES9457236, and OREF 592-12A.