REPARATIVE TISSUES IN ARTICULAR CARTILAGE DEFECTS IN A CANNE MODEL TREATED BY MICROFRACTURE

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INTRODUCTION

The recently introduced technique of microfracture (µfx) for the arthroscopic treatment of articular cartilage (AC) defects involves limited scraping of the calcified cartilage and multiple puncturing of the subchondral plate. Initial reports of the clinical findings have been promising. The objective of this study was to evaluate the tissue types comprising the reparative tissues formed in AC defects in a canine model treated by: a) µfx alone; b) µfx + type II collagen implant (CI); and, for comparison, c) autologous chondrocyte-seeded CI. All treatments were applied using an open procedure. The principal tissue type in all of the defects was fibrocartilage. The µfx-treated defects with the CI contained 40% more tissue than the lesions receiving µfx alone, but the difference did not reach statistical significance.

METHODS

Twelve adult dogs, 25-30 kg were used in the study. Two 4-mm diameter defects were produced in the trochlear groove of the right knee of the animals as previously described [1]. All AC was removed and the calcified cartilage surface scraped with a customized curette. In animals receiving cultured autologous chondrocytes, AC was harvested from the lateral and medial margins just outside the trochlear groove of the left femur. The cells were isolated and expanded in culture for 3 weeks and seeded into a type II CI (Chondrocell, Geistlich Biomaterials, Wolhusen, Switzerland) that was previously described [2]. Cell seeding of matrices was performed between 4 and 12 hours before implantation.

In 16 defects in 8 dogs, µfx was performed with the straight pointed end of a microsurgical pick to a depth of approximately 1.5-2.0 mm. The width of the pick at this depth was approximately 0.8 mm. Four holes were produced in each lesion. Eight of these defects received no additional treatment, and 8 received the CIs covered by a type II collagen film. The collagen film, produced in our laboratory from a slurry of the Chondrocell material, was sterilized and cross-linked by UV radiation. The collagen film covering the CI was sutured to the adjacent AC. In the remaining 4 animals that did not receive µfx, chondrocyte-seeded CIs were placed into the 8 defects. The implants were covered by the type II collagen film that was sutured to the adjacent AC. Operated knees were immobilized by external fixation for 10 days. Animals were sacrificed 15 weeks after treatment. The animal experiment was approved by the Brockton/West Roxbury VA Animal Care Committee.

Paraffin sections were stained with H & E, trichrome, Safranin O/ fast green, and antibodies to types I and II collagen. One section from the middle portion of each defect was analyzed to determine the areal percentage of specific tissue types [1]: fibrous tissue, fibrocartilage, or hyaline cartilage. The histomorphometric analysis was restricted to the area of the original defect; it did not include reparative tissue formed in regions below the tidemark.

RESULTS

Animals in all groups appeared to ambulate normally at the time of sacrifice. Grossly, there were no signs of inflammation in any of the dogs. Histomorphometrically, type II matrix-implanted µfx-treated lesions were filled to the top of the defect; however, the trend toward more total filling was not found to be statistically significant ($p = 0.20$; comparing the µfx and µfx + CI groups; see Table 1). Although the reparative tissue was predominantly fibrocartilage in these two groups (Table 1), there was more Safranin O and type II collagen staining in the matrix-implanted group. None of the groups displayed more than 2% hyaline cartilage. The reaction in the bone of the matrix-implanted group was much greater than in the group with µfx alone. The deeper portions of the defect contained more cartilaginous tissue than the surface, staining at least lightly for Safranin O throughout. Hyaline cartilage was found at the periphery of the defect above the small section of calcified cartilage that was not resorbed.

The cell-seeded CI group also displayed an extensive reaction in the underlying bone in many samples. There was intense staining for Safranin O, mostly at the level of the underlying bone, but extending somewhat into the defect area; this finding was more consistent in the µfx + CI group than in the other groups.

Damage in the adjacent cartilage from suturing included mechanical disruption, tissue forming in the suture track, and near complete loss of Safranin O staining from the matrix.

Table 1. Areal percentage of types comprising the reparative tissues in the three experimental groups.

		Defect Filling (Percent, mean ± SEM)			
Treatment	**n**	**Total Filling**	**Fibrous Tissue**	**Fibro-Cartilage**	**Hyaline Cartilage**
µfx	8	64 ± 18	11 ± 4	52 ± 8	1 ± 0.3
µfx + CI	8	90 ± 9	17 ± 9	72 ± 12	2 ± 1
Cell-Seeded CI	8	70 ± 16	15 ± 7	54 ± 19	1 ± 0.2

DISCUSSION

In work previously reported [3], the areal percentage of tissue filling in untreated defects in the canine model was approximately 30%. Fifteen weeks postoperatively, approximately 16% of the reparative tissue was hyaline cartilage. Thus, while µfx doubled the total amount of tissue filling the defect, it yielded virtually no hyaline cartilage. Longer term follow-up will be required to determine the fate of the fibrocartilage in the µfx-treated groups, with and without the CI. Previous reports [1, 3] have described the chronology of reparative tissues in defects treated with cultured autologous chondrocytes under a periosteal cover in this animal model. Defects, predominately filled with hyaline cartilage but with a total filling of less than 50% after 3 months, displayed degenerative changes and less hyaline cartilage by 18 months. The question is whether defects with almost complete filling with fibrocartilage will fare better longer term than defects filled only 50% by hyaline cartilage.

The results of this study showed no benefit of a chondrocyte-seeded type II CI over a µfx-treated defect in which an unseeded CI was implanted. Future work will be needed to determine the optimal characteristics of a chondrocyte-seeded CI.

ACKNOWLEDGMENT

This work was supported by the Harvard/MIT Division of Health Sciences and Technology and the Department of Defense (HAB), the BioKinetix Foundation, and the Brigham Orthopedic Foundation.

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