Hyaluronic Acid Hydrogels for Articular Cartilage Defect Repair in a Large Animal Model

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Introduction

Intrinsic repair of articular cartilage damage is unsatisfactory. Due to limitations of current surgical treatments,[1] tissue engineering (TE) approaches have been pursued as promising alternatives. Our laboratory investigates hyaluronic acid (HA) hydrogels as a scaffold for cartilage TE.[2-6] When seeded with mesenchymal stem cells (MSCs) and cultured in defined media containing the chondrogenic factor transforming growth factor-β3 (TGF-β3) for several months, these constructs can reach near native biomechanical and biochemical properties.[4,5] As opposed to pre-maturing these constructs prior to implantation, an alternative approach could be to encapsulate cells in HA hydrogels along with TGF-β3 laden microspheres[5] (all polymerized in-situ) within a defect to induce differentiation and direct new matrix production in-vivo. Delivering such a high dose of TGF-β3 can induce cartilaginous matrix production in both in-vitro and subcutaneous in-vivo models.[5,6] Our long-term goal is to compare these approaches directly within a clinically relevant in-vivo cartilage injury model. As a first step, the goal of this study was to assess the natural healing response to an HA hydrogels alone or delivering TGF-β3 in a large animal model of cartilage injury and repair.

Methods

In four Yucatan minipigs, full thickness chondral defects (4 mm diameter) were created in the trochlear groove of the stifle joint. In each animal, four experimental groups were compared: 1) an untreated defect (n=8), 2) a methacrylated HA hydrogel (1% w/v), polymerized in situ using UV light (n=4), 3) a HA hydrogel, laden with alginate microspheres containing TGF-β3 (50 ng) (n=4), as previously described,[5] and 4) a cartilage autograft transfer (CAT) (n=4). Normal cartilage served as a positive control. At 6 weeks, animals were euthanized. Bone morphometry under the defect site was determined using microcomputed tomography (μCT). Bone volume per total volume (BV/TV) was calculated for the first 2 mm underneath the defect. Histological evaluation included cell morphology (hematoxylin & eosin) and matrix composition (proteoglycan and collagen staining via Alcian blue/picrosirius red and Safranin O/fast green). Samples were scored using a modified ICRS-II system.[7] BV/TV between groups was compared via ANOVA with Bonferroni post-hoc tests ($p<0.005$). For histological scoring, individual comparisons were made using the Mann-Whitney test ($p<0.005$).

Results

Six weeks after surgery, bone morphology of the treated groups featured evidence of bone remodeling and resorption beneath the defects (Fig. 1A). The largest changes in BV/TV were observed in the HA/MS group, which was 72% lower than normal controls (Fig. 1B, $p<0.005$). In terms of histologic appearance (Fig. 2), the untreated group filled incompletely with a mostly fibrous tissue with diffuse staining for type I collagen, but little for type II collagen. HA treatment led to considerable variability, with some samples featuring robust staining for proteoglycans and type II collagen, while others contained more fibrous tissue. The HA/MS group displayed a mostly fibrous appearance and substantial bone remodeling. Nevertheless, marked type II collagen was found within these defects. The CAT group filled the vast majority of the defect space, stained well for proteoglycans, and contained type II collagen; however, these constructs integrated poorly with the surrounding tissue. In terms of ICRS-II scoring, the median overall values for the untreated group were 62% lower than normal (Fig. 3A, $p<0.005$). In terms of defect fill, the untreated, HA, and HA/MS groups were all significantly lower than normal (Fig. 3B, $p<0.005$). No statistically significant differences were detected between the CAT and normal groups ($p>0.005$). Similar results were found in terms of matrix staining (Fig. 3C). The HA group had the highest variability, with two scores above 70%. All experimental groups were significantly different from normal controls in terms of integration to the surrounding cartilage (Fig. 3D, $p<0.005$). Interestingly, the CAT group had the lowest median scores, which were under 50% of normal ($p<0.005$).

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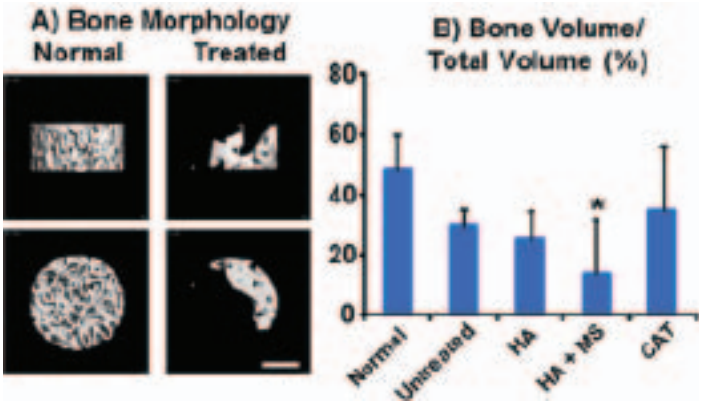

Figure 1A-B. Morphometric analysis of subchondral bone. 3-D μCT reconstructions of bone from normal and experimental specimens (A) (centered under defect, scale bar = 0.5mm). Bone Volume/Total Volume (B) (*p<0.005 vs. normal).

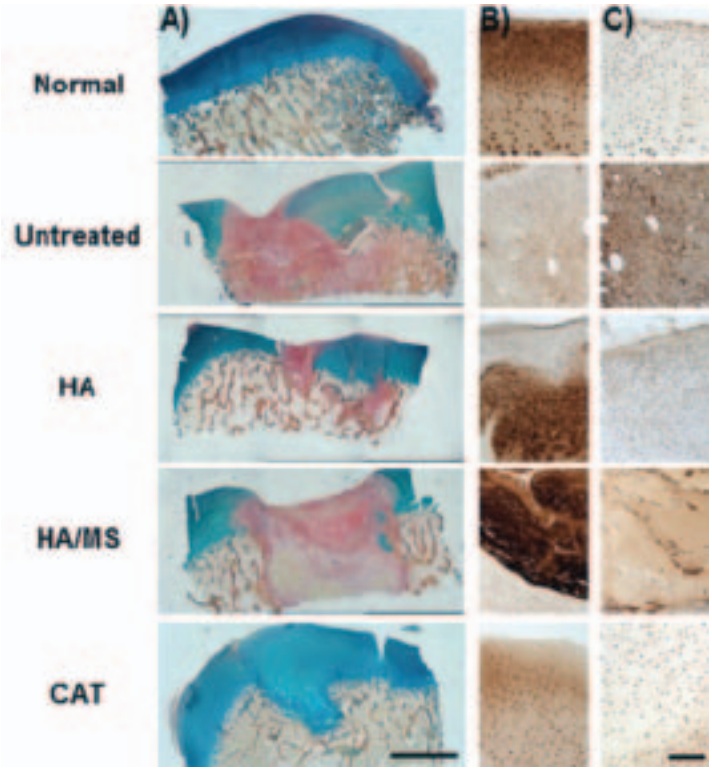

Figure 2. Histological staining for proteoglycans (blue) and collagen (red) following 6 weeks in-vivo (A) (scale=2mm). Immunohistochemical staining for type II (B) and type I (C) collagen.

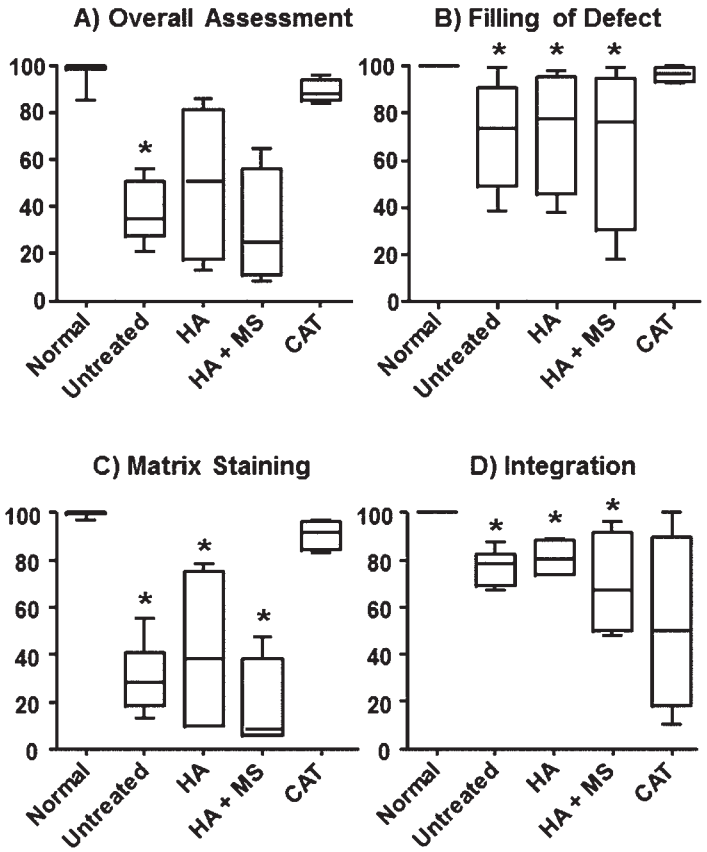

Figure 3A-D. ICRS-II scoring: Overall assessment (A), filling of defect (B), matrix staining (C), and integration to surrounding cartilage (D) (*p<0.005 vs. normal).

Discussion

In this preliminary study, we compared the healing response to HA and TGF-β3 to an untreated defect and a chondral autograft within a porcine model of cartilage injury and repair. Untreated defects were unable to heal spontaneously with cartilage-like tissue by 6 weeks. Alternatively, autologous cartilage autografts were able to fill the defect, but had poor integration to the surrounding cartilage. These results match those observed clinically.[1] Interestingly, HA alone had some positive impact on tissue healing, with increased proteoglycan and type II collagen staining relative to untreated defects. However, these results were highly variable. The addition of microspheres containing TGF-β3 increased type II collagen staining within the defect; however, little proteoglycan staining was found, and substantial subchondral bone remodeling occurred. This indicates a complex response to TGF-β3 within this model, and suggests its use must be carefully controlled in future studies. Longer-term studies are also warranted to determine the full time course of healing following these treatments. Since the healing tissue with HA treatment appeared to be well-colonized with cells, this system will allow us to determine whether extrinsically supplied MSCs or those that migrate to the injury site provide a better outcome. If cells need to be provided, we will also test whether pre-maturation of MSC-seeded HA constructs or direct encapsulation and implantation provides superior outcomes for cartilage repair.

Significance

The positive, but limited, results with HA alone or HA with microspheres delivering TGF-β3 for cartilage repair suggest that additional factors (e.g. cells) are needed to fully restore the articular cartilage following injury in this model. These data will guide future work in developing cell-laden tissue engineered constructs for cartilage repair.

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