Introduction: Many articular cartilage (AC) repair procedures have recently been introduced into the clinic. One involves the harvesting of AC for autologous cell implantation. Concerns over the effects that this and other repair techniques may have on the AC throughout the joint prompted this study. In particular, we hypothesized that changes may occur in the physical and biochemical properties of uninvolved AC adjacent to the harvest sites. Thus, our objective was to quantify site-dependent changes in cartilage stiffness, streaming potential, thickness, glycosaminoglycan (GAG), DNA and water content in a canine knee model for chondral defect repair. Significant changes, up to 300%, were found in some physical and biochemical properties at certain sites following selected surgical procedures 15-18 wks post-operatively.

Materials and Methods: The right and left knees from 12 canines were evaluated. This animal experiment was approved by the Brockton/West Roxbury VA Animal Care Committee. Two 4-mm diam. defects, down to the tidemark in the right trochlear groove of adult dogs, were treated with microfracture (4x, n=4 dogs), microfracture plus type II collagen implant (C.I., 4), or autologous cell-seeded CI (CSCI, 4). Chondrocytes for CSCI were isolated from the trochlear ridges of 5 left knees 3 wks prior to treatment of the defects; cells from one joint were not used. The remaining 7 left knees served as unoperated controls. Animals were sacrificed 15 wks post-operatively and 9.5-mm diam. osteochondral cores were taken at sites shown in Figure 1. Sites damaged during retrieval were discarded; undamaged specimens were stored at -20°C until testing. The AC and underlying bone surrounding the core was allocated for histology.

On the day of testing, the osteochondral core was thawed at room temperature in phosphate buffered saline (PBS) with proteinase inhibitor. The underlying bone was mounted in self-curing PMMA. The mounted specimen was placed in a testing chamber clamped into a Dynatrac mechanical spectrometer. An indentation protocol using a 1-mm plane-ended Plexiglass indenter equipped with an Apf/ApCl electrode was employed, in part, in anticipation of future studies of biphasic material. After rewelling in PBS and equilibration with a 0.01 kg tare load, two sequential step loads (±10 and 15% strain) were applied and the equilibrium loads recorded. Sinusoidal displacements (strain amplitude ±1%) were superimposed on the 15% static strain at frequencies ranging from 0.005 to 1.0 Hz and the resulting loads and streaming potentials recorded. A final step displacement (total strain ±20%) was applied and the equilibrium load recorded. AC thickness was then measured using a needle-tap method. The equilibrium Young's modulus of the tissue was computed using the analysis of Hayes, et al. from equilibrium stress (σ = load/indenter area) and strain (ε = displacement/measured thickness).

After mechanical testing, half the AC was removed from cores from 7 of the 30 test sites. This AC was weighed wet, lyophilized, weighed dry, and digested in pepsin. The remaining AC and underlying bone was allocated for histology. Papain digests were analyzed for total GAG (by DMB dye binding assay) and DNA (by the diphenylamine reaction). GAG and DNA content were normalized by dry weight of the undigested tissue. Tissue allocated for histology was fixed in formalin, decalcified in EDTA, dehydrated in a paraffin or 9°4. Micrometioned sections were stained with Safranin/fast green. Results are presented as percent increases or decreases from control (mean ± SEM). Differences were assessed using a two-tailed unpaired Student's t-test between surgical and control groups.

Results: No gross abnormalities in the AC from any of the joints were evident at necropsy. However, there were significant (p<0.05) changes in some of the mechanical and biochemical properties. The largest and most significant changes were seen in the dynamic properties (Table 1). Normalized streaming potential in the harvest group increased as much as 300% above control values (p<0.001). No significant changes (p>0.05) in dynamic properties were noted for the CI group. Although there were no significant changes in thickness, there were significant (p<0.05) increases in water content at the AMF in the harvest, CI and nfx groups. Other significant increases in biochemical content were seen in DNA/dw at CMT in harvest and CSCI groups, and GAG/dw at PMT in the CI group. Many more changes, including changes in thickness and modulus, were significant at p<0.10. No obvious changes were found in histological sections from tissue showing the greatest alterations in mechanical properties.

Discussion: Although there were no gross changes in the appearance of the AC, differences were seen in mechanical and biochemical properties at all sites except ALF. Most intriguing were the dramatic increases in dynamic stiffness and streaming potential of uninvolved AC adjacent to harvest sites compared to unoperated controls. These observations are suggestive of changes seen in hypertrophic remodeling noted in some animal models of early OA. Being covered or uncovered by the meniscus did not affect changes. No correlations were seen between changes in mechanical and biochemical properties. Clearly, further studies are necessary to determine how these changes will affect long-term performance. Moreover, this study was conducted in canines and it is not clear that the same changes would be seen in humans. However, it is important to be aware of the potential affects that certain surgical treatments may have on human AC.


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HARVEST AND SELECTED CARTILAGE REPAIR PROCEDURES AFFECT MECHANICAL AND BIOCHEMICAL PROPERTIES OF UNINVOLVED ARTICULAR CARTILAGE IN THE CANINE KNEE

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