Effects on Exposed Articular Cartilage During Open Surgical Procedures: A Comparison of Various Fluids in an Animal Model

Jack Farr, M.D., Leanne M. Mathew, B.S., Aaron M. Stoker, M.S., Ph.D., Seth L. Sherman, M.D., and James L. Cook, D.V.M., Ph.D.

Purpose: The aim of this study was to assess the potential detrimental effects of the operating room environment on exposed healthy articular cartilage and to evaluate tissue hydration treatment strategies for preserving chondrocyte viability and extracellular matrix composition in this environment. **Methods:** With institutional Animal Care and Use Committee approval, femoral and tibial condyles (n = 36; 6 per specimen) were harvested from canine cadavers (n = 6) immediately after euthanasia and placed on a draped operating table under standard surgical lighting for a timed 2-hour period. Each condyle was randomly assigned to one of 6 groups (n = 6 per group): no-treatment control, hyaluronic acid (HA), saline sponge, saline drip, culture media (Dulbecco's modified Eagle's medium [DMEM]) sponge, or culture media drip. Full-thickness cartilage sections were collected from each specimen immediately after harvest (time 0) and immediately after 2-hour exposure (time 2H), and processed to determine chondrocyte viability, tissue water content, and extracellular matrix composition (glycosaminoglycan [GAG] and collagen content). **Results:** Chondrocyte viability was significantly lower ($P = .03$) after the 2-hour exposure in the control group. HA, saline sponge, and saline drip treatment groups all had significantly higher ($P < .043$) chondrocyte viability compared with controls at time 2H. Water content was significantly lower ($P < .01$) after the 2-hour exposure in the control group. Further, the water content in the control group was significantly lower than all treatment groups at time 2H ($P < .001$). No significant differences in tissue collagen or GAG content were observed within groups between time points or among groups at either time point. **Conclusions:** Canine articular cartilage did not demonstrate any reduction in chondrocyte viability or tissue water content at 2 hours when treated with hyaluronic acid, saline drip, saline-soaked sponge, or DMEM-soaked sponge compared with untreated exposed cartilage. **Clinical Relevance:** Surgeons should consider the use of a hydrating solution for the treatment of exposed articular cartilage during open joint surgery of 2 hours or longer duration.

Focal cartilage defects often require surgical treatments involving open arthrotomy, exposing the joint to the operating room environment. Areas of cartilage that are not undergoing treatment during these procedures are often neglected, which may result in chondrotoxic effects from dehydration or desiccation, or both.[1-5] Protection of exposed cartilage that is not undergoing treatment is of critical importance in that the success of the surgical procedure and long-term function of the patient is dependent on maintaining the overall health of the joint. The importance of protection of articular cartilage during surgery also applies to donor cartilage that is exposed during osteochondral allografting procedures.[6]

Hydration of exposed tissues is a basic surgical principle that is taught to surgeons, but it can prove

From Indiana University School of Medicine, OrthoIndy Cartilage Restoration Center (J.F.), Indianapolis, Indiana; Comparative Orthopaedic Laboratory (L.M.M., A.M.S., J.L.C.), and Department of Orthopaedic Surgery, Missouri Orthopaedic Institute (S.L.S., J.L.C.), University of Missouri, Columbia, Missouri, U.S.A.

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Address correspondence to James L. Cook, D.V.M., Ph.D., Comparative Orthopaedic Laboratory and Missouri Orthopaedic Institute, University of Missouri, 1100 Virginia Ave, DC953.00, Columbia, MO, 65212 U.S.A. E-mail: cookjl@health.missouri.edu

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clinically impractical or simply be forgotten because of the technical detail of the primary procedure. Previous studies recommended hydrating exposed cartilage with lactated Ringer solution every 10 to 20 minutes to optimally preserve chondrocyte viability.[3] Although physiological isotonic solutions such as lactated Ringer solution or saline provide a readily available and cost-effective method for hydrating exposed cartilage in the operating room, the recommended frequency of application is high,[3] and substances specifically directed toward maintaining articular cartilage health may be more optimal for this purpose. For example, media formulated for culture and preservation of hyaline cartilage is associated with maintenance of chondrocyte viability and matrix integrity.[7,8] Similarly, hyaluronic acid (HA) has been reported to effectively provide chondroprotective effects for hyaline cartilage.[1,5,9-12] Culture media and HA are readily available for use in surgical procedures. Therefore, investigating their capabilities for protection of healthy articular cartilage exposed to operating room conditions has clinical merit.

The purpose of this study was to assess the potential detrimental effects of the operating room environment on exposed healthy articular cartilage and to evaluate tissue hydration treatment strategies for preserving chondrocyte viability and extracellular matrix composition in this environment. We hypothesized that healthy articular cartilage would experience significant losses in cell viability and water content after a 2-hour exposure and that all tissue hydration treatments would be associated with preservation of chondrocyte viability and extracellular matrix composition (water, glycosaminoglycan [GAG], and collagen contents) so that none of these measures of tissue health would be significantly reduced after a 2-hour exposure.

Methods

Tissue Harvest

All procedures were approved under the Institutional Animal Care and Use Committee's general policies and procedures for the use of canine cadaveric tissues. Femoral and tibial condyles were harvested from canine cadavers (n = 6) immediately after euthanasia was performed for reasons unrelated to this study. Condyles were harvested en bloc (n = 36) as follows: six 6 cadavers × 2 stifles (knees) × 4 condyles per knee (medial and lateral femoral and medial and lateral tibial) = 48 condyles available, of which we used 36 for the study because one condyle from each knee (randomly chosen) was used for a concurrent unrelated study. Each harvested condyle was assigned a number and was placed on a draped operating table under standard surgical lighting in a temperature-controlled (20°C to 23°C), humidity-controlled (35% to 45%), laminar air-flow 1,800 square-foot room for a timed 2-hour period. Full-thickness cartilage sections were collected from each specimen (n = 2 per specimen per time point) immediately after harvest (time 0) and immediately after 2-hour exposure (time 2H), and processed to determine chondrocyte viability, tissue water content, and extracellular matrix composition, as described further on. Each condyle was randomly assigned to one of the following groups (n = 6 per group):

- Control: Condyles were left exposed to the operating room environment without hydrating treatment of any type
- HA: 0.5 mL of Synvisc Hylan G-F 20 (Genzyme, Ridgefield, NJ) was applied and manually spread over the entire articular surface of the condyle at time 0
- Saline sponge: Two 4 × 4 gauze sponges were completely saturated with phosphate-buffered saline (PBS) and applied at time 0 so that they covered the entire articular surface of the condyle for the 2-hour test period
- DMEM sponge: Two 4 × 4 gauze sponges were completely saturated in DMEM (Gibco, Grand Island, NY) and applied at time 0 so that they covered the entire articular surface of the condyle for the 2-hour test period
- Saline drip: 5 mL of PBS was applied by dripping the solution over the entire articular surface of the condyle using a pipette, beginning at time 0 and repeating every 15 minutes for the 2-hour test period
- DMEM drip: 5 mL of DMEM was applied by dripping the solution over the entire articular surface of the condyle using a pipette, beginning at time 0 and repeating every 15 minutes for the 2-hour test period

Chondrocyte Viability

The live and dead cells in cartilage from each condyle at each time point were identified using the commercially available fluorescent Live/Dead Viability/Cytotoxicity Kit (Life Technologies, Grand Island, NY) following manufacturer guidelines. Tissues were exposed to the stains for 30 minutes at room temperature, rinsed for 5 minutes in PBS, mounted for fluorescence microscopy, and assessed using an Olympus BX51 microscope (Olympus, Essex, England). Images were taken at ×4 magnification, and green-staining live cells and red-staining dead cells were counted using a custom in-house cell counting program. Percent chondrocyte viability (% CV) was calculated and reported as percent cell viability using the equation: % CV = [total live cells/(total live cells + total dead cells)] × 100.

Tissue Water Content

Cartilage from each condyle was weighed (g) immediately after collection (wet weight) and after