

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

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**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.

**F**ocal 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.<sup>1-5</sup> 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.<sup>6</sup>

Hydration of exposed tissues is a basic surgical principle that is taught to surgeons, but it can prove clinically impractical or simply be forgotten because of the technical detail of the primary procedure. Previous

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studies recommended hydrating exposed cartilage with lactated Ringer solution every 10 to 20 minutes to optimally preserve chondrocyte viability.<sup>3</sup> 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,<sup>3</sup> 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.<sup>7,8</sup> Similarly, hyaluronic acid (HA) has been reported to effectively provide chondroprotective effects for hyaline cartilage.<sup>1,5,9-12</sup> 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  $\times$  2 stifles (knees)  $\times$  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  $\times$  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  $\times$  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  $\times 4$  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)]  $\times$  100.

### Tissue Water Content

Cartilage from each condyle was weighed (g) immediately after collection (wet weight) and after lyophilization (dry weight). Percent water content (% WC) at time 0 and time 2H was determined using the equation:

% WC = (sample wet weight – sample dry weight)/ sample wet weight.

### Extracellular Matrix Analysis

Lyophilized cartilage tissue samples from each condyle at each time point were digested with papain overnight at 60°C. The result was then used to determine GAG and collagen content of the tissue using the dimethylmethylene blue<sup>13</sup> and hydroxyproline (HP) assay<sup>14</sup> as previously described. The determined GAG and HP contents were standardized to the tissue dry weight to account for variations in tissue size.

### Statistical Analysis

Data from each outcome measure at each time point (time 0 and time 2H) were compared among treatment groups using a one-way analysis of variance and Tukey post hoc test. Data within each treatment group were compared between time 0 and time 2H using a Student *t* test. Significance was set at  $P < .05$ .

## Results

### Chondrocyte Viability

Percent chondrocyte viability (Fig 1) was significantly lower ( $P = .03$ ) after the 2-hour exposure in the control group (no hydration) compared to time 0. No significant differences in % CV between time 0 and time 2H were noted within any of the tissue hydration treatment groups. The HA, saline sponge, and saline drip treatment groups all had significantly higher ( $P < .043$ ) % CV compared with control at time 2H. Additionally, at time 2H the HA, saline drip, saline sponge, and

DMEM sponge treatment groups all had significantly higher ( $P < .043$ ) % CV than the DMEM drip group.

### Tissue Water Content

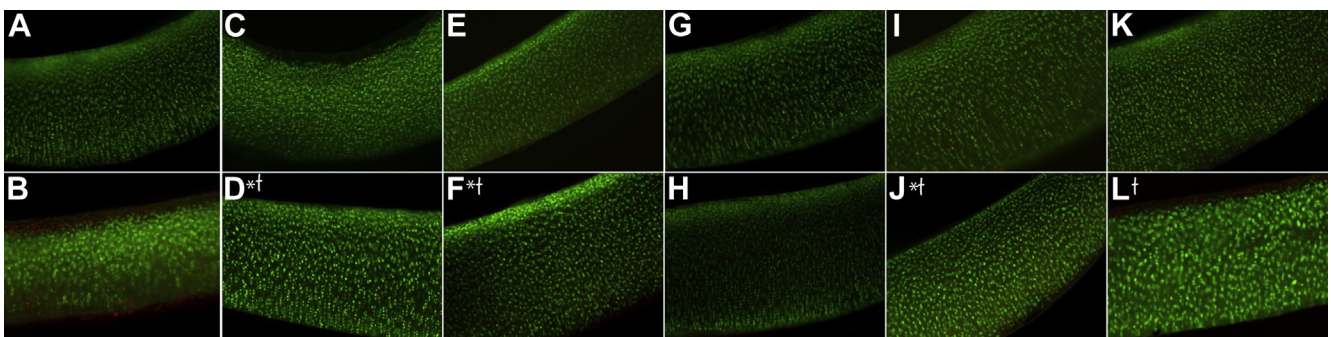
Percent water content (Table 1) was significantly lower ( $P < .01$ ) after the 2-hour exposure in the control group (no hydration) compare to time 0. Further, the % WC in the control group (no hydration) was significantly lower than all treatment groups at time 2H ( $P < .001$ ). No other significant differences in % WC were noted within groups between time points or among groups at either time point.

### Extracellular Matrix Biochemistry

No significant differences in tissue collagen (HP) or GAG content were observed within groups between time points or among groups at either time point (Table 1).

## Discussion

The key result from this study is that application of HA or a hydrating solution such as saline to healthy cartilage during an open surgical procedure may prevent the potentially detrimental changes in articular cartilage cell viability, water content, GAG content, and collagen content that occur as a result of a 2-hour exposure to the operating room environment. This study indicates that healthy canine articular cartilage undergoes significant decreases in chondrocyte viability and water content in as little as 2 hours of exposure to operating room conditions. In our experience, articular cartilage is often exposed for this amount of time during cartilage grafting, articular fracture, and other common open joint surgical procedures and may be exposed for



**Fig 1.** Representative images of live (green) and dead (red) cells in cartilage samples taken at 4× magnification: (A) control group (no treatment) at time 0 and (B) 2-hour exposure (time 2H); (C) hyaluronic acid (HA) at time 0 and (D<sup>\*†</sup>) at time 2H; (E) saline drip at time 0 and (F<sup>\*†</sup>) at time 2H; (G) media drip at time 0 and (H) at time 2H; (I) saline sponge at time 0 and (J<sup>\*†</sup>) at time 2H; and (K) media sponge at time 0 and (L<sup>†</sup>) at time 2H. After images were taken, 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. The viability of the cartilage samples decreased significantly ( $P = .03$ ) from the time 0 to time 2H time point in the no-treatment group. There was not a significant decrease in the viability of any of the other treatment groups from time 0 to time 2H. The saline sponge, saline drip, and HA treatment groups had significantly higher viability at time 2H compared with the no-treatment group.

\*Samples with significantly higher cell viability than the time 2H no-treatment control group ( $P < .043$ ).

†Samples possessed significantly greater viable cell counts than time 2H DMEM drip ( $P < .043$ ).

**Table 1.** Summary of Data

Group	% CV		% WC		GAG ( $\mu\text{g}/\text{mg}$ dry weight)		HP ( $\mu\text{g}/\text{mg}$ dry weight)	
	Time 0	Time 2H	Time 0	Time 2H	Time 0	Time 2H	Time 0	Time 2H
Control	86 $\pm$ 2	71 $\pm$ 7 <sup>†</sup>	72 $\pm$ 1	55 $\pm$ 2 <sup>†</sup>	807 $\pm$ 258	1,056 $\pm$ 183	43 $\pm$ 14	48 $\pm$ 16
HA	87 $\pm$ 2	90 $\pm$ 1*	75 $\pm$ 3	75 $\pm$ 2*	1298 $\pm$ 375	1084 $\pm$ 278	54 $\pm$ 17	46 $\pm$ 13
Saline Drip	87 $\pm$ 1	90 $\pm$ 2*	69 $\pm$ 2	75 $\pm$ 2*	1021 $\pm$ 209	916 $\pm$ 316	31 $\pm$ 14	33 $\pm$ 18
DMEM Drip	83 $\pm$ 3	78 $\pm$ 4	72 $\pm$ 1	70 $\pm$ 3*	1069 $\pm$ 346	677 $\pm$ 184	33 $\pm$ 16	35 $\pm$ 21
Saline Sponge	84 $\pm$ 3	87 $\pm$ 2*	69 $\pm$ 2	75 $\pm$ 2*	1084 $\pm$ 354	791 $\pm$ 232	47 $\pm$ 16	41 $\pm$ 13
DMEM Sponge	88 $\pm$ 1	86 $\pm$ 5	74 $\pm$ 1	74 $\pm$ 2*	1106 $\pm$ 191	739 $\pm$ 171	47 $\pm$ 13	52 $\pm$ 16

NOTE. Percent cell viability (% CV), percent water content (% WC), tissue proteoglycan (GAG), and tissue collagen (HP) data presented as  $\pm$  standard error. The water content of the control no-treatment group at 2-hour exposure (time 2H) was significantly lower ( $P < .01$ ) than the control no-treatment group at time 0, and all other treatment groups at time 2H. There was no significant difference in the GAG and HP content of any group between time 0 and time 2H or between any of the treatment groups.

DMEM, Dulbecco's modified Eagle's medium; HA, hyaluronic acid.

\*Samples significantly higher than the time 2H no-treatment control ( $P < .001$ ).

<sup>†</sup>Groups with significantly lower ( $P < .043$ ) value at time 2H than at time 0.

much longer periods during more complicated procedures, highlighting the critical importance of protection of this tissue. To optimize successful outcomes for these procedures, it is vital that the viability and composition of exposed cartilage that is not undergoing treatment are maintained, especially in light of the limited healing capacity of articular cartilage.<sup>3,5</sup>

In the present study, application of HA or hydration with saline was effective in preserving chondrocyte viability, tissue hydration, and the extracellular matrix for a 2-hour exposure period. A previous study reported that hydration of cartilage with lactated Ringer solution every 10 or 20 minutes significantly decreased human chondrocyte death after a 2-hour exposure compared with cartilage receiving no hydration.<sup>5</sup> Taken together with our data, it is apparent that the frequency of rehydration plays a crucial role in determining chondrocyte survival when using a drip method. For clinical application of a drip-hydrating method, this frequency requirement would need to be addressed by surgeons and operating room personnel, who are intensely focused on the primary procedure being performed, so that the necessary time and attention is given to ensure these frequent applications of hydrating solutions. Although application of hydrating solutions to exposed articular cartilage through a saturated sponge was effective for both saline and DMEM in this study, this method would also appear to be problematic in practice. It requires continuous coverage of the cartilage with the saturated sponge, which would likely pose a physical obstacle to surgeons and therefore be unlikely to receive compliance. A single application of HA on initial exposure of the tissue was as effective as the other hydrating solutions tested in preventing potentially detrimental changes in articular cartilage that occur as the result of a 2-hour exposure to the operating room environment in this study. However, financial and availability aspects regarding the use of HA may be impediments for its use in this application.

## Limitations

This study includes an ex vivo experimental design using canine tissues that were not matched pairs and the use of a single 2-hour assessment time point in comparison to time 0. In addition, clinical variables such as blood in the operative site and manipulations of adjacent tissues were not evaluated. Although these limitations certainly should be considered when interpreting and applying the data, we consider ex vivo studies to be the most ethical method for studying this clinical question, and the single 2-hour time point was felt to be most practical and applicable so that sufficient numbers of specimens could be directly compared with the specimens available. Certainly, earlier and later time points for assessment, as well as additional hydration application frequencies, would be additive for determining time-dependent responses and potential longer term effects of changes in cell viability, water content, and extracellular matrix composition. The current study design was felt to optimally use available canine tissues and funding and limit variability and risk for type II errors in statistical analyses. Based on the inclusion of time 0 assessments, a valid control, controlled operating room environmental conditions, and head-to-head comparisons, we are confident in the applicability of these data as stated.

## Conclusions

Canine articular cartilage did not show any reduction in chondrocyte viability or tissue water content at 2 hours when treated with HA, saline drip, saline-soaked sponge, or DMEM-soaked sponge compared with untreated exposed cartilage. Therefore, protection of articular cartilage with a hydrating solution during open joint surgery appears to be critical for preserving chondrocyte viability and tissue water content, both of which are known to be vital for cartilage health. This principle could also be applied to donor cartilage that is exposed during osteochondral allografting procedures.

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