

Preservation of Unaffected Cartilage During Open Surgical Procedures



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Introduction

Focal cartilage defects often require surgical treatments involving open arthrotomy, exposing the entirety of the joint to the surrounding environment. Unaltered cartilage during these procedures is often neglected, causing potential damage to these tissues. In an effort to reduce the negative effects that accompany open surgical procedures, we designed this study to assess the extent of damages through cell viability and water content testing of the tissues after exposure to a surgical setting. In addition, we attempted to discover the best strategy possible for maintaining cartilage health during these surgical procedures.

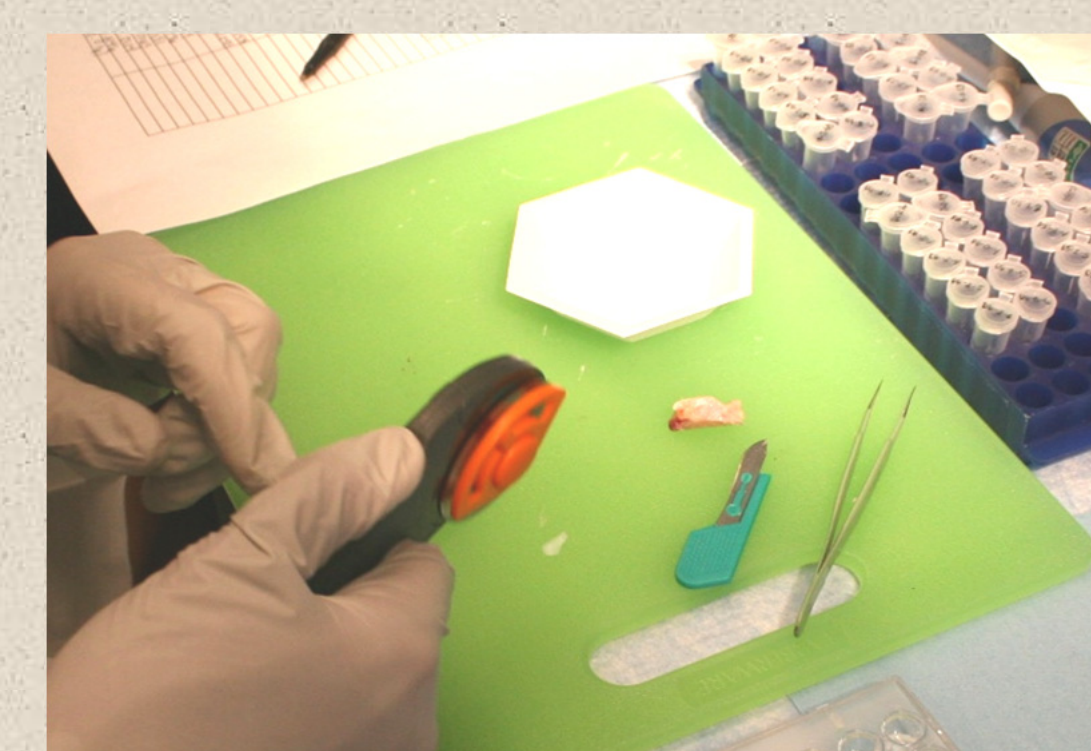
Objective

To determine an optimal method of cartilage preservation during open surgical procedures

Methods

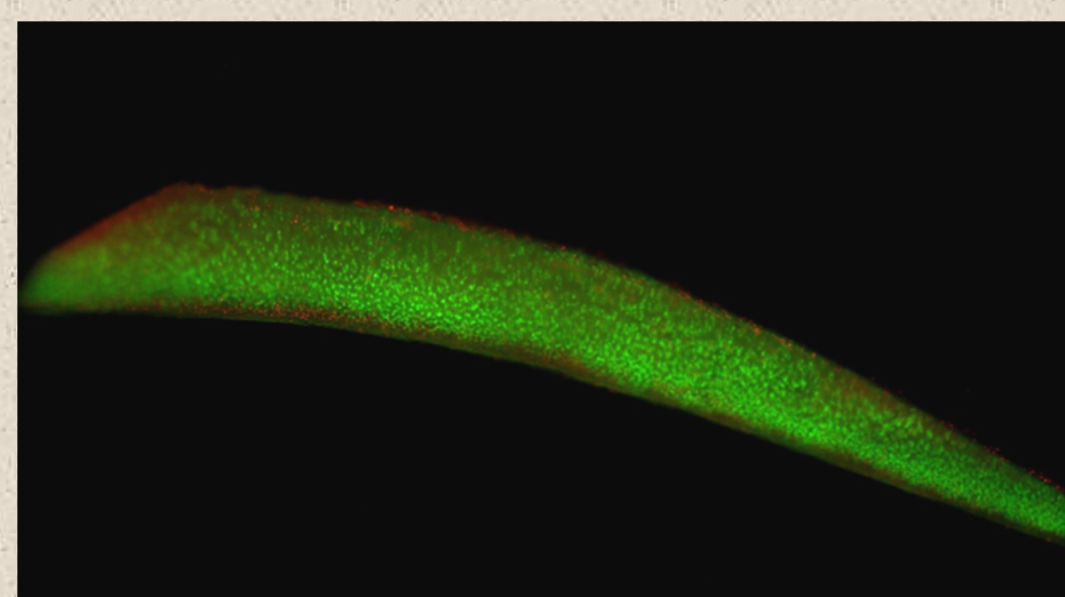
All procedures were approved by the IACUC and the animals used were euthanatized for reasons unrelated to this study.

Tissue Harvest: Femoral condyles (FC) and tibial plateaus (TP) were dissected from canines (n=10) en bloc, and each surface (FC or TP) was placed in one of the following treatment groups: 1) Control – No Treatment, 2) Hyaluronic Acid (HA), 3) Phosphate Buffered Saline (PBS) Sponge, 4) Dulbecco's modified Eagle's medium (DMEM) Sponge, 5) PBS Drip, or 6) DMEM Drip. Each treatment was carried out for 2 hours.



Tissue Processing: Cartilage samples were collected from each surface immediately after dissection (Time 0) and post-treatment (Time 2H) and processed for cell viability analysis, tissue water content, and extracellular matrix composition.

Cell Viability: Samples were analyzed for viability using fluorescent viability dyes calcein AM and ethidium homodimer, and images of live and dead chondrocytes were collected via microscopy. Live and dead cells were counted using a computer algorithm and percent cell viability ($\frac{\text{\#live cells}}{\text{\#total cells}} \times 100$) was calculated.



Water Content: Samples were weighed (g) immediately after dissection (initial weight) and placed into corresponding tubes to be lyophilized overnight. Samples were weighed (g) again post-lyophilization (final weight). Percent water for Time 0 and Time 2H samples ($\frac{[\text{sample weight initial} - \text{sample weight final}]}{\text{sample weight initial}}$) was determined.

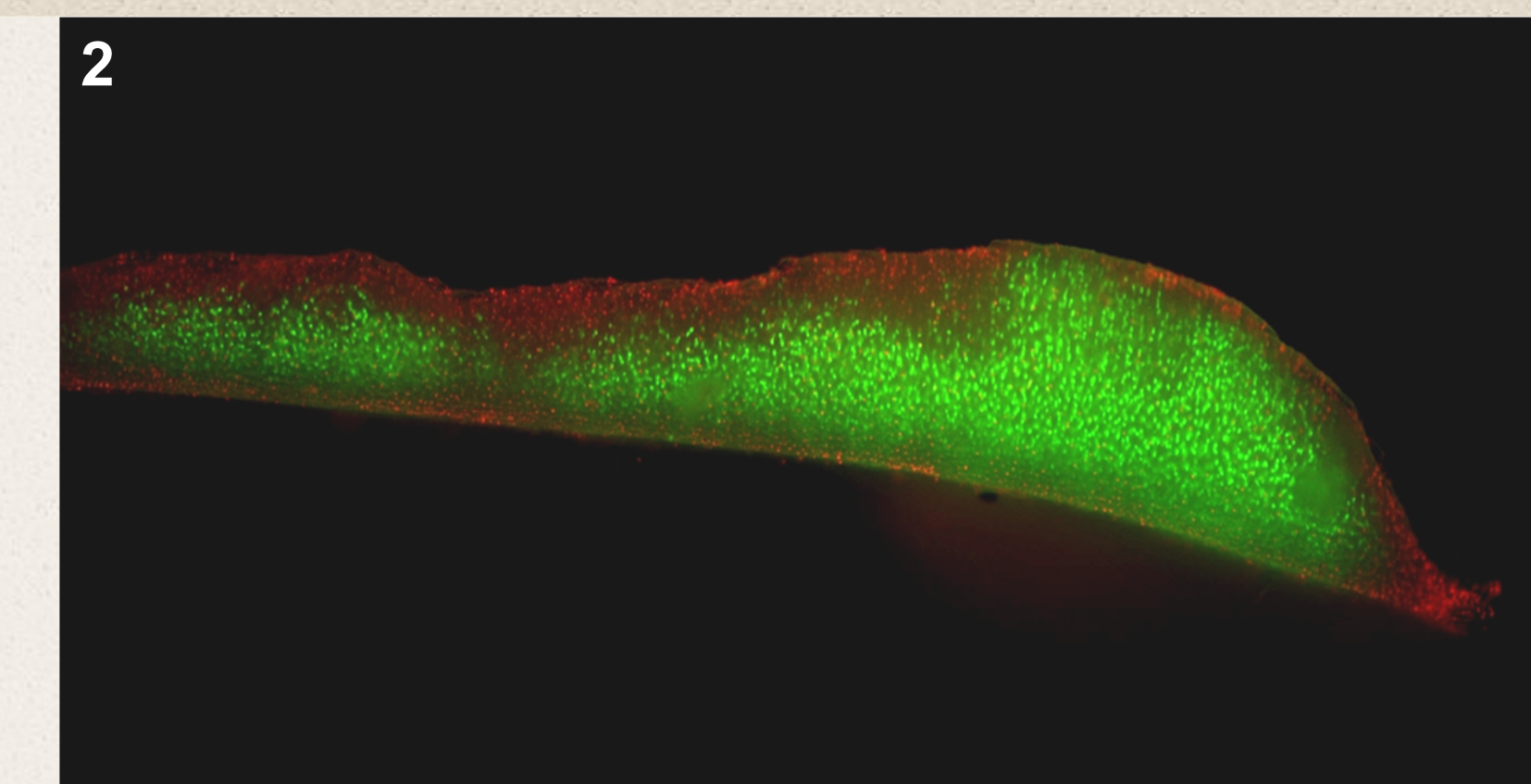
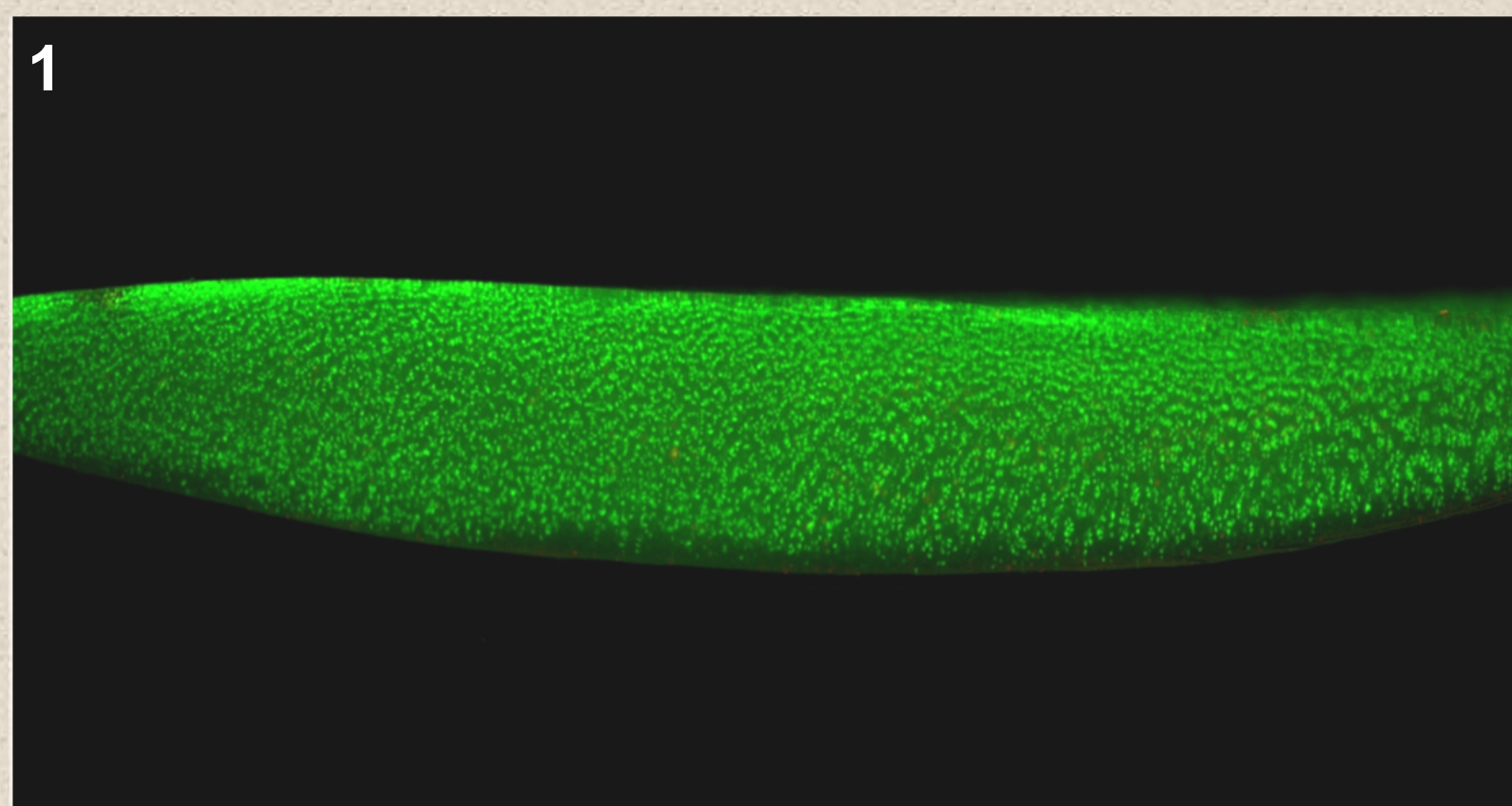
GAG & HP Analysis: Lyophilized samples were digested using 1mL of papain digestion solution to measure glycosaminoglycans (GAG)—dimethylmethylene blue assay—and collagen—hydroxyproline (HP) assay—content of tissues.

Statistical Analysis: Treatment comparisons and Time 0 versus Time 2H comparisons were performed with SigmaPlot® using ANOVA followed by t-Test with significance set at $p < 0.05$.

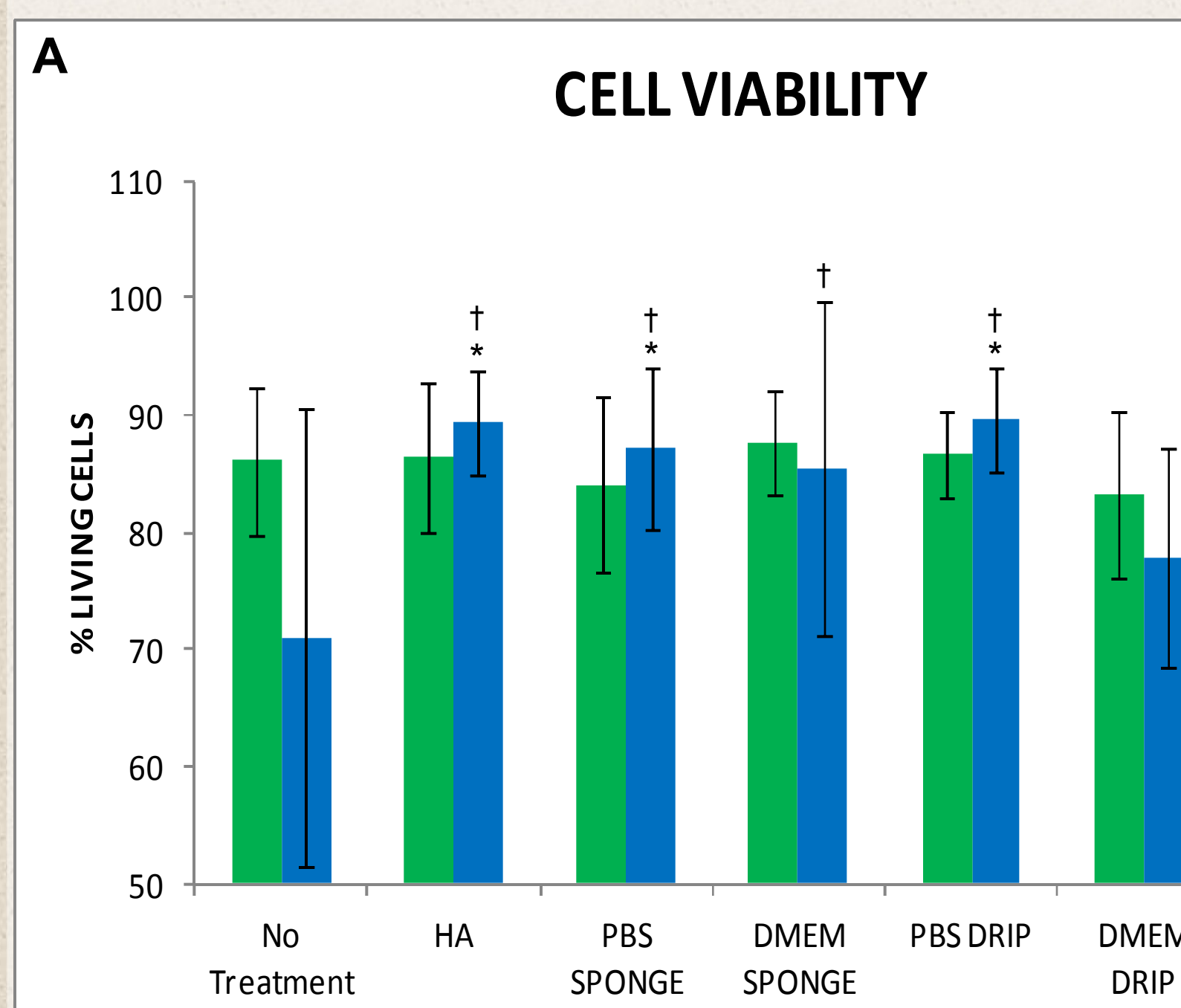
Discussion

These data indicate that exposing unaltered cartilage in a surgical setting can detrimentally impact health and moisture, even in a short amount of time. Clinically, if surgeons were to hydrate unaltered cartilage during surgical procedures with HA or PBS, this could prevent unnecessary damage due to cell death and dehydration from occurring. The extracellular matrix of cartilage would not be altered by application of these treatments, as shown by our HP and GAG assays. By providing a source of moisture during surgical procedures, surgeons can increase a treatment's overall success by reducing the negative effects of exposing a joint on untreated cartilage surfaces of the joint.

Figures



1 & 2) Representative images of live (green) and dead (red) cells taken at 4x magnification of Control – No Treatment Time 0 (Fig. 1) and Time 2H (Fig. 2) cartilage samples.

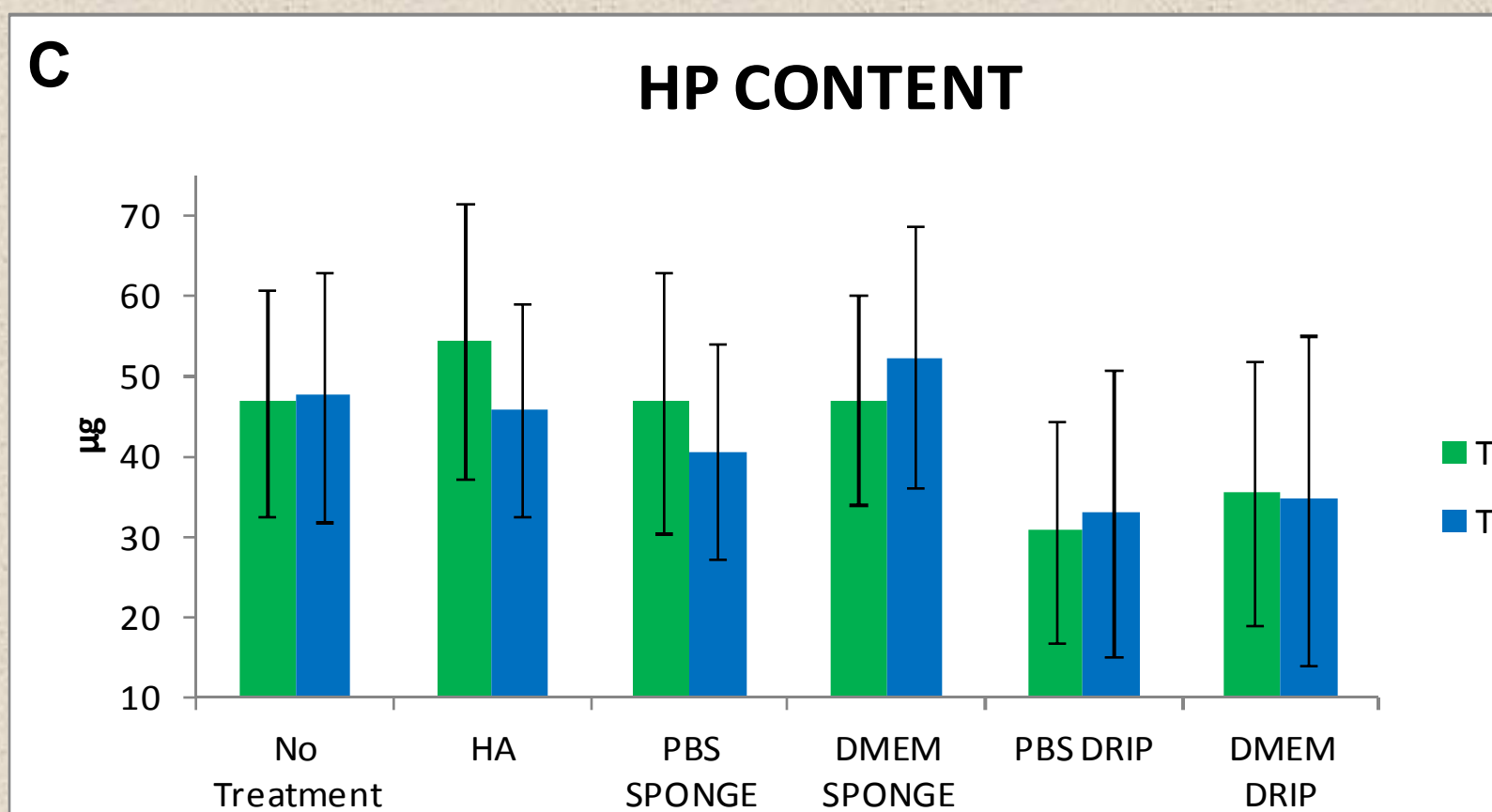
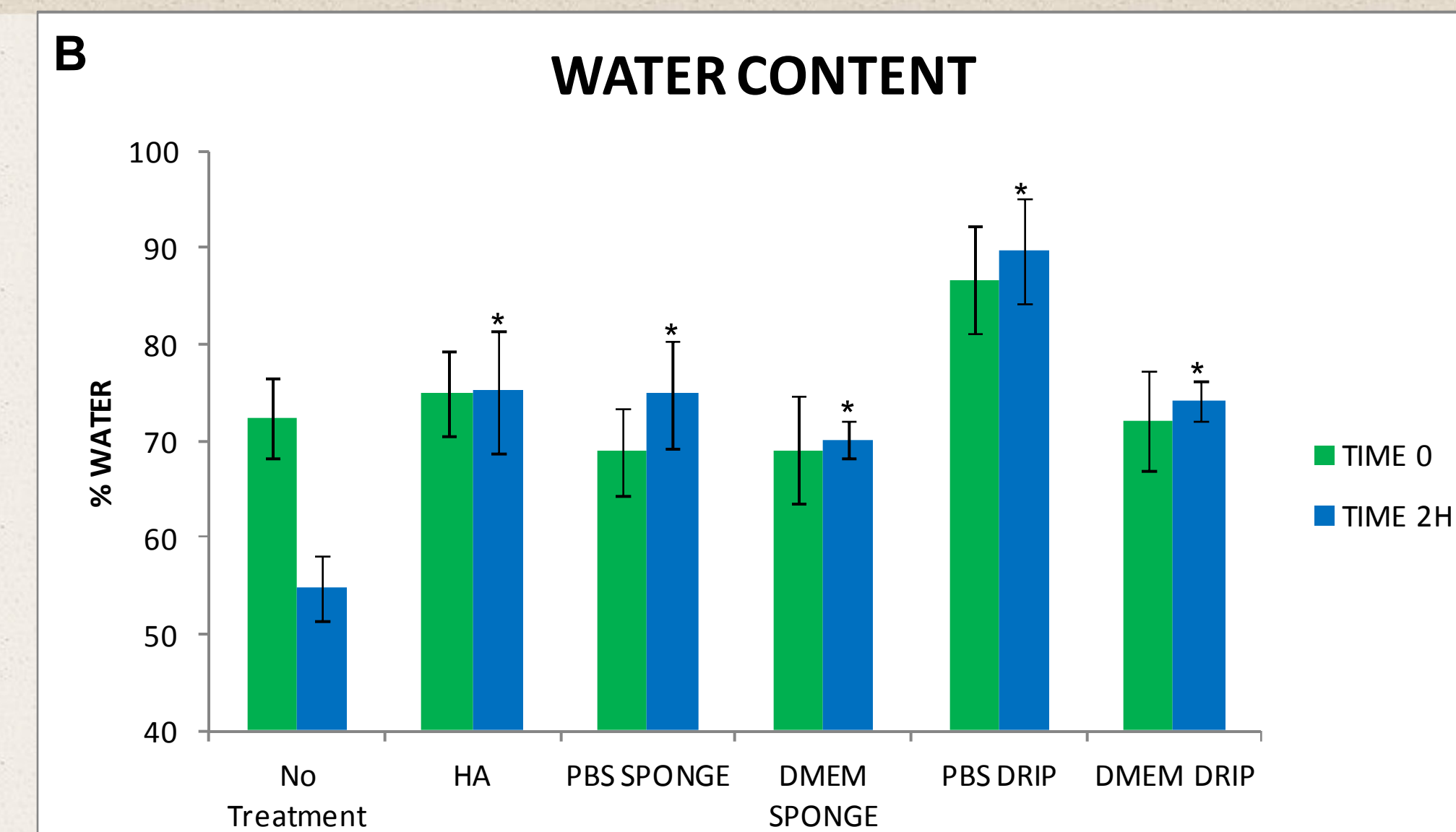


A) The viability of the cartilage samples decreased significantly from Time 0 to the 2 hr time point in the no treatment group. There was not a significant decrease in the viability of any of the other treatment groups from Time 0 to Time 2H. However, many of the treatment groups had significantly higher viability at Time 2H compared to the no treatment group.

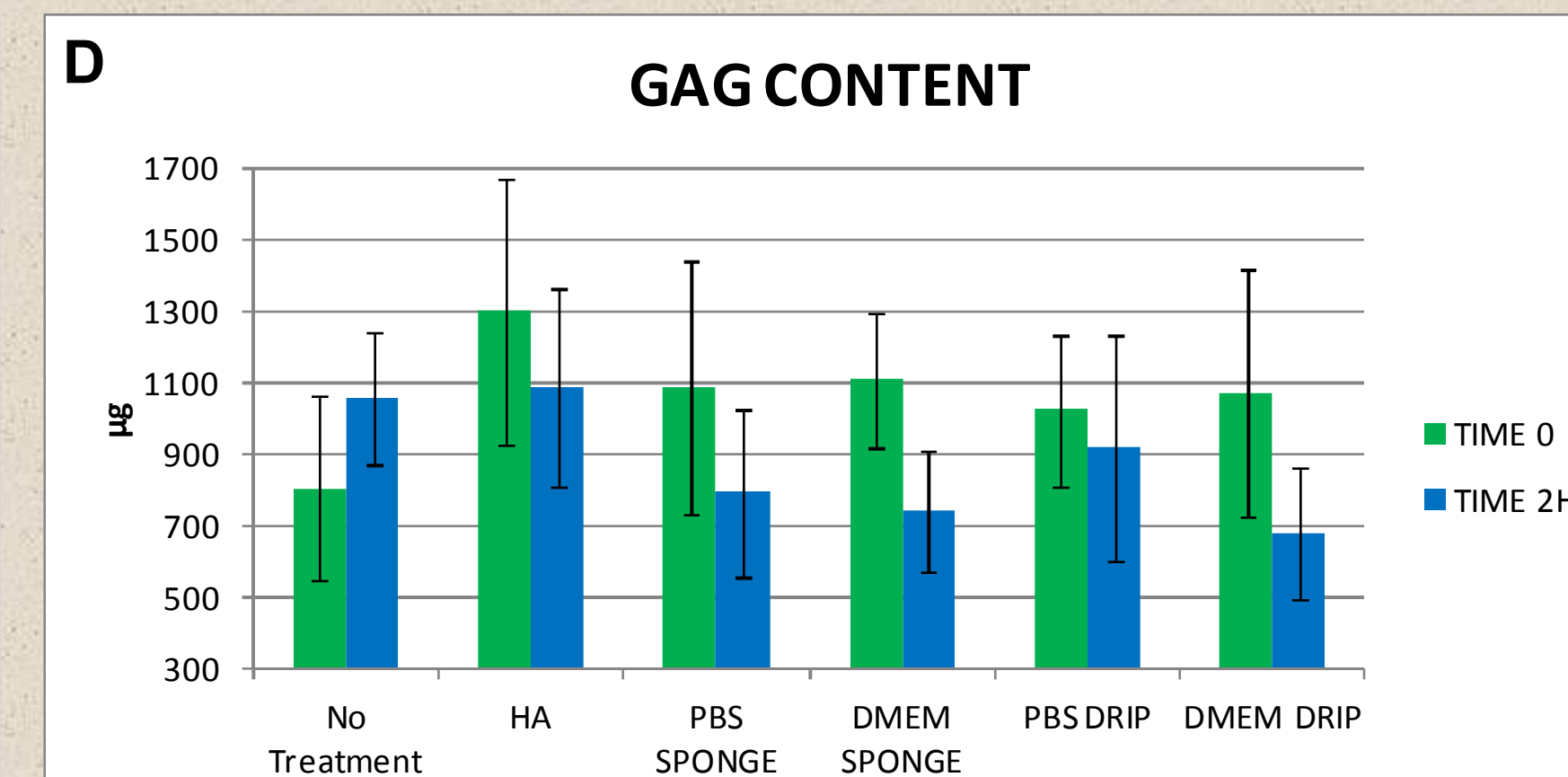
(*) Samples exhibited significantly higher living cell count than Time 2H Control – No Treatment ($p < 0.05$). (†) Samples possessed significantly greater living cell counts than Time 2H DMEM Drip ($p < 0.05$).

B) The percent water content of the cartilage tissue decreased significantly from the time 0 to the 2hr time point in the no treatment groups. There was not a significant decrease in water content from time 0 to the 2hr time point for any of the treatment groups.

*Samples demonstrated significantly higher water content than Time 2H Control – No Treatment ($p < 0.05$).



C) The collagen content of the cartilage tissue was not significantly ($p > 0.05$) different between time points for any of the groups analyzed.



D) The proteoglycan content of the tissue was not significantly ($p > 0.05$) different between time points for any of the groups analyzed.

Conclusions

- Not providing hydration to the cartilage tissue during open arthrotomy can cause a decrease in tissue hydration and cell viability which can have detrimental lasting effects on the health of the tissue and the joint.
- Providing hydration to the cartilage tissue using HA and PBS treatments during open arthrotomy can help to maintain tissue hydration and cell viability during the surgical procedure.

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