Hyperosmolar Stress: Investigating Articular Cartilage Response Using an Ex Vivo Injury Model

Unterguggenberger C[1,2], Jahangir S[1], Salzmann GM[2,3], Stoddart M[1], Grad S[1,], Schmal H[2,4], Kubosch EJ[2,*]*

[1] AO Research Institute Davos, Switzerland.

[2] Department of Orthopedics and Trauma Surgery, Medical Center-University of Freiburg, Freiburg, Germany.

[3] Schulthess Klinik, Zürich, Switzerland.

[4] Department of Orthopaedic Surgery, University Hospital Odense, Odense, Denmark.

Introduction: Physiological 0.9% saline is commonly used as an irrigation fluid in modern arthroscopy. Recent investigations indicate that hyperosmolar saline solutions possess chondroprotective effects, particularly when confronted with iatrogenic injury, opening a promising avenue for improved cartilage preservation. This study aimed to comprehensively explore the multifaceted response of articular cartilage to hyperosmolar stress and mechanical injury. Specifically, the objectives were two-fold: (1) to characterize the spatial chondrocyte survival conferred by hyperosmolar saline solution post-injury and (2) to unravel the modulatory response of articular cartilage to combined osmotic stress and mechanical trauma.

Methods: A Controlled Laboratory Study was designed to understand the complex interaction between osmotic conditions, physical injury, and the chondrocytes response. Osteochondral explants derived from bovine stifle joints were methodically subjected to two distinct conditions: exposure to 0.9% physiological saline (308 mOsm) or a hyperosmolar saline solution (600 mOsm). Following exposure for 20 minutes, a precisely delineated full-thickness cartilage injury was induced using a sharp dermatome blade. The explants were then maintained in the respective fluids for an additional 3 hours before being transferred to a chondro-permissive medium, where they underwent one week- culture. Chondrocyte survival was quantified through live/dead staining and confocal imaging. Simultaneously, the cellular response was tracked over the course of a week by analysing relative gene expressions pertaining to apoptotic and inflammatory markers, coupled with the release of Glycosaminoglycans (GAGs) and Nitric Oxide (NO) into the culture medium. Data was generated from 10 separate experiments, each carried out using osteochondral explants from two stifle joints of one animal. Statistical analysis was performed using GraphPad Prism (GraphPad Software, CA, USA). One-way ANOVA was executed and a post-hoc Tukey's test. The significance threshold was set at $p < 0.05$.

Results: Evident from the results was the manifestation of a confined Zone of Cell Death resulting from the full-thickness cartilage incision, primarily affecting chondrocytes in the superficial zone. Notably, samples exposed to hyperosmolar saline demonstrated significantly curtailed expansion of cell death in both axial ($p = .007$) and coronal planes ($p = .004$). Surprisingly, during the following week of culture (on day 7), there was a noteworthy lack of ongoing cell death for both control and hyperosmolar saline groups. Histological assessments with Safranin O & fast green staining showed the preservation of the cartilage matrix integrity, coupled with maintenance of normal chondrocyte morphology. Moreover, analysis of gene expressions implicated initial upregulation (on day 1 and 3) of inflammatory (IL6 and NOS2) and proapoptotic (BAX) genes, subsequently transitioning to pronounced downregulation by the seventh day. Furthermore, GAGs and NO release into the culture medium exhibited a concentration peak on the third day.

Conclusion: In sum, this in-depth exploration utilizing an in vitro cartilage injury model not only underscores the chondroprotective potential of hyperosmolar saline irrigation fluid but also sheds light on the impressive capacity of normal articular cartilage to rapidly restore joint homeostasis following the tandem challenges of osmotic stress and mechanical injury.

Significance/Clinical Relevance: Raising the osmolarity of an irrigating fluid emerges as a straightforward and safe maneuver with the potential to profoundly impact articular cartilage preservation during arthroscopic interventions, thereby offering a clinically valuable insight.

Key words: cartilage, irrigation, osmolarity, injury, chondroprotection, arthroscopy