The Acidity of Normal Saline Irrigation Impairs Chondrocyte Health by Promoting Oxidative Stress

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INTRODUCTION: For decades, surgeons have utilized 0.9% normal saline (NS) for joint irrigation to improve visualization during arthroscopic procedures. This continues despite mounting evidence that NS exposure impairs chondrocyte metabolism and compromises articular cartilage function. NS differs from synovial fluid in terms of its low pH, hypotonicity, and lack of glucose, any of which could contribute to its toxicity. While this indicates a need for a more biocompatible joint irrigant, it is unclear how this can be achieved without better knowledge of the mechanisms underlying NS toxicity. The primary goal of this study was to define the mechanism of NS toxicity in an effort to develop a practical intervention that can be rapidly and widely adopted for clinical use. We hypothesized oxidative stress induced by low pH is the dominant factor driving NS toxicity and that buffering NS to increase its pH would help mitigate these effects.

METHODS: Bovine stifle joints were procured from a local abattoir for isolation of primary bovine chondrocytes and synoviocytes. Monolayer and 3D agarose cultures were used to investigate the effects of irrigants on cell viability, morphology, and oxidant production using fluorescent probes and confocal microscopy. Sterilized 0.9% NS and culture media served as a control for all experiments, and 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), a zwitterionic buffering agent, was supplemented to NS to determine the effects of pH regulation. Cell viability was assessed by incubating primary bovine chondrocytes in culture media and normal saline for 3 hours with subsequent staining with CellTiter 96 reagent and quantification using a microplate reader. Changes in cellular morphology were verified by phalloidin immunofluorescence staining and confocal microscopy. Oxidant production was assessed by co-staining samples with Calcein AM and dihydroethidium (DHE) to visualize live cells and reactive oxygen species (ROS) production respectively under confocal microscopy. Metabolomic analysis was completed on treated samples using high-resolution mass spectrometry following gas and liquid chromatography. All quantified data was normalized to culture media control. Data was analyzed by one-way ANOVA with the Tukey post-hoc test using SPSS Statistics. Statistical significance was set at $p < 0.05$.

RESULTS: Cell viability of NS at 3 hours was 43.5 ± 13.9% ($p < 0.001$) compared to control. This cytotoxicity was validated by morphological changes, with severe cytoskeletal damage resulting in dramatic cell shrinkage and detachment in chondrocytes incubated in NS. Chondrocytes treated with NS had increased production of ROS as imaged by DHE staining (Figure 1). The levels of ROS after 3 hour incubation were approximately 500-fold ($p = 0.03$) and 2.4-fold ($p < 0.001$, Figure 1) higher than control in both monolayer and 3D agarose culture systems, respectively. Supplementation of 25 mM HEPES in 0.9% NS significantly reduced cellular damage characterized by phalloidin staining. The addition of HEPES significantly reduced ROS levels in both monolayer culture ($p = 0.033$) and 3D agarose culture ($p = 0.012$, Figure 1). Bovine synoviocytes exposed to NS also demonstrated a similar trend of ROS overproduction with a 3-fold ($p = 0.002$) increase compared to control with HEPES supplementation negating this deleterious effect ($p < 0.001$). Exposure to NS caused disruption of glycolysis, pentose phosphate and tricarboxylic acid pathways and buffering with HEPES helped return metabolite levels to near control levels (Table 1).

DISCUSSION: In the present study, NS exposure induced ROS production in chondrocytes and synoviocytes in addition to cell shrinkage, detachment, and death. Metabolomic analysis clearly showed that NS exposure profoundly disrupted metabolic pathways related to energy production, intracellular signaling, and antioxidant defenses. The addition of HEPES to NS significantly reduced ROS production and cytotoxicity and restored metabolic function to near control levels, supporting the hypothesis that the sub-physiologic pH of NS is at least partly to blame for its negative effects on chondrocytes. This harmful effect, in conjunction with other forms of injury that can occur during arthroscopy including iatrogenic injury and the loss of lubricin, a critical boundary lubricant, from the articular surface could lead to significant injury to chondrocytes and synovium in an already damaged joint. With arthroscopy used increasingly in young and healthy patient populations, the cumulative effects of this damage may be difficult to quantify in the immediate postoperative period; however, these adverse effects may contribute to delayed recovery or increase the risk of osteoarthritis and potential arthroplasty in patients who have previously undergone arthroscopy.

SIGNIFICANCE/CLINICAL RELEVANCE: While NS remains a commonly utilized irrigation solution in arthroscopic surgery, our data suggests that exposure to unbuffered NS profoundly disrupts articular cartilage and synovial cell function which can be reversed through the addition of HEPES, a readily available biologic buffer.

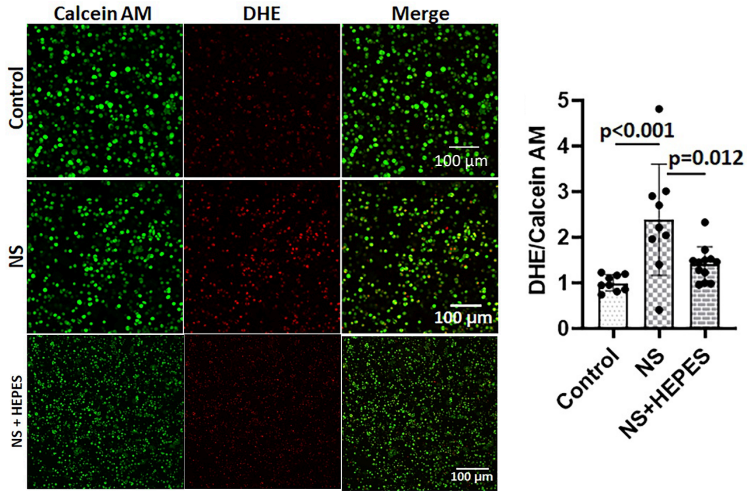

Figure 1: Representative confocal images of DHE and Calcein AM in 3D agarose culture after 3 hours (left) and quantified DHE/Calcein AM ratios normalized by control group (culture media) (n = 9-12, right).

	NS *versus* Control		NS + HEPES *versus* Control	
Metabolites (up)	**Fold change**	**p-value**	**Fold change**	**p-value**
Glucose 6-phosphate	70.3	5.9E-09	23.4	1.5E-04
Ribose 5-phosphate	34.3	5.8E-09	6.8	1.2E-02
Fructose 6-phosphate	7.5	5.9E-09	2.8	7.6E-04
D-Ribulose 5-phosphate	4.2	5.8E-09	2.0	1.6E-05
Adenosine monophosphate (AMP)	2.3	5.8E-09	0.8	9.1E-03
Metabolites (down)	**Fold change**	**p-value**	**Fold change**	**p-value**
Reduced nicotinamide adenine dinucleotide phosphate (NADPH)	-31.1	6.6E-09	-0.8	7.2E-04
Alpha-Ketoglutarate (KG)	-22.0	5.8E-09	-15.5	5.8E-09
Alpha-Ketoisovalerate (KIV)	-12.4	6.1E-09	-4.8	8.3E-09
Cytidine triphosphate (CTP)	-11.8	5.8E-09	-1.4	5.7E-06
Nicotinamide adenine dinucleotide + hydrogen (NADH)	-9.6	1.2E-07	-0.8	7.0E-03
Lactate	-7.2	5.8E-09	-3.3	5.8E-09
Oxidized glutathione (GSSG)	-4.8	7.9E-08	-1.7	1.4E-04

Table 1: List of metabolites showing ≥ 2-fold changes between control and 0.9% normal saline (NS). Chondrocytes were treated with each irrigation solution for 30 minutes (n = 6). Positive fold change (up): NS or NS + HEPES > control, negative values change (down): NS or NS + HEPES < control.