Introduction: Chondrocyte-matrix interactions help maintain the unique biomechanical properties of articular cartilage (1). Mechanical injury results in chondrocyte death and matrix degradation (2). Articular cartilage defects heal poorly, with the fibrocartilaginous repair tissue contributing to joint incongruity, progressive damage within native cartilage surrounding the lesion and eventual cartilage degeneration (2,3). Minimising chondrocyte death from mechanical injury is beneficial, as it may help retain a cell population within the defect capable of restoring a normal hyaline architecture and avoid the inherent problems with fibrocartilagenous repair that have limited a multitude of experimental and therapeutic attempts at cartilage healing (2). 0.9% Saline and Hartmann’s are commonly used joint irrigating solutions during articular surgery (4). We hypothesized that chondrocyte death in mechanically wounded articular cartilage is decreased by increasing the osmolarity of joint irrigating solutions.

Materials and Methods: The osmolarity of 0.9% Saline (285 mOsm) and Hartmann’s (255 mOsm) solutions was varied from 100-600 mOsm by the addition of distilled water or sucrose. Osteochondral explants (rectangular blocks, n=72) harvested from the metacarpophalangeal joints of three-year old cows (N=6) were exposed to the solutions of different osmolarity for 2 minutes to allow in situ chondrocytes to respond to the altered osmotic environment. Explants were then wounded through the full thickness of articular cartilage with a fresh scalpel and incubated (37°C, 5%CO₂) in the same solution for 2.5 hours. Cell viability was determined using the fluorescent probes, Chloromethylfluorescein diacetate (stains cytoplasm of live cells green) and Propidium iodide (stains nuclei of dead cells red) and low power (x10) confocal laser scanning microscopy (CLSM). Percentage cell death (PCD; 100 x number of dead cells/ number of dead and live cells) was quantified using the fluorescent probes, Chloromethylfluorescein diacetate (stains cytoplasm of live cells green) and Propidium iodide (stains nuclei of dead cells red) and low power (x10) confocal laser scanning microscopy (CLSM). Percentage cell death (PCDFT) was quantified within a ROI measuring 971x200x60 μm³ (x-y-z axes, respectively). For z-sections acquired perpendicular to the z-axis (imaging only the superficial zone below the articular surface), percentage cell death (PCDFT) was quantified at 100 μm intervals from the articular surface downwards within a ROI measuring 971x100x60 μm³ (x-y-z axes, respectively). For z-sections acquired parallel to the z-axis (imaging all zones within the full thickness of cartilage at the wound edge), percentage cell death (PCDSZ) was quantified at 100 μm intervals from the articular surface downwards within a ROI measuring 971x100x60 μm³ (x-y-z axes, respectively). PCDFT and PCDSZ were compared between explants exposed to solutions of varying osmolarity.

Results: PCDFT confirmed that cell death was localised to the superficial zone (~ first 100 μm from the articular surface) of wounded cartilage for the range of medium osmolarities (100-600 mOsm), with relative sparing of the middle and deep zones (analysis of variance (ANOVA), p<0.05). Compared to the control explants exposed to 0.9% Saline (285 mOsm), PCDSZ was greatest for the low osmolarity (100 mOsm) saline solution and least for the high osmolarity (600 mOsm) saline solution (ANOVA, p=0.04, Figure 1).

Discussion: Increasing the osmolarity of 0.9% Saline and Hartmann’s solutions is chondroprotective in a surgically relevant model of cartilage wounding. These experiments have important clinical relevance for the design of irrigation solutions during arthroscopic and open articular surgery. Surprisingly, routinely used Hartmann’s solution was associated with additional chondrocyte death compared to 0.9% Saline, with the difference not completely accounted for by the lower osmolarity of the Hartmann’s solution. We hypothesize this may be due to the lower calcium concentration in joint irrigating solutions merits further investigation.


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Figure 1: Chondrocyte death at the wound edge decreases with increasing osmolarity of the saline solution (* p<0.05 compared to control)

Figure 2: Decrease in chondrocyte death at the wound edge by increasing the osmolarity of Hartmann’s (p<0.05 for paired comparisons, Figure 2).