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Efficacy of common surgical compounds in preventing articular chondrocyte death from desiccation

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Abstract

Purpose Purpose of this study was to identify potential substances that prevent desiccation of chondrocytes.

Methods Macroscopically normal bovine cartilage explants ($n = 80$) were exposed to room air, or covered with surgical lubricant, Lactated ringer (LR) or Sefrafilm (Genzyme Biosurgery, Cambridge, MA) for 0, 30, 60 or 120 min. The viability of superficial chondrocytes was measured after 48 h of incubation in tissue culture media at 37 °C by Live/Dead staining. Chondrotoxicity was measured as the extent of cell death below the articular surface. Statistical analysis was performed with a two-way analysis of variance on the data set and a subsequent Tukey's post hoc test.

Results Chondrocyte death correlated positively with the length of exposure, regardless of the treatment ($p < 0.0001$). The extent of superficial chondrocyte death was minimally lower in the LR ($89.1 \pm 2.6 \%$, $80.8 \pm 1.2 \%$) and surgical lube ($84.3 \pm 1.8 \%$, $75.9 \pm 2.7 \%$) groups than the control ($82 \pm 5.7 \%$, $65.6 \pm 13.3 \%$) and Sefrafilm group ($77.6 \pm 3.9 \%$, $63.3 \pm 6.9 \%$) ($p < 0.001$) at the first two time points, with

no significant difference between the latter groups. After 60 and 120 min, surgical lube resulted in less chondrocyte death than all other groups ($70.4 \pm 6.8 \%$ and $60.9 \pm 5.9 \%$, all $p < 0.0001$).

Conclusion The data suggest that depending on the expected length of exposure of the articular cartilage surface, different compounds appear to be protective. For exposures exceeding 60 min, surgical lubricant demonstrated the highest protective potential. Results from this study indicate that protecting exposed articular surfaces with surgical lubricant for orthopaedic procedures lasting more than 1 h lead to decreased chondrocyte death and suggest improved cartilage functional outcomes postoperatively.

Keywords Chondrotoxicity · Desiccation · Surgical compounds · Arthrotomies · Cartilage

Introduction

Various orthopaedic procedures, ranging from fracture fixation to joint preservation/reconstruction, require arthrotomies with joint surfaces exposed to room air. It is known that articular cartilage exposed for prolonged periods of time results in dehydration that is chondrotoxic and may lead to joint degeneration. [1, 8, 13, 16] Previous studies have demonstrated that as little as 30 min of desiccation of articular cartilage results in significant chondrocyte death. Periodic rewetting with lactated ringer (LR) solution has been recommended every 10–20 min to prevent chondrocyte death [13]. Hyaluronan, a component of synovial fluid, has also been previously studied as a cartilage anti-desiccant and has been reported to be effective [1, 7, 9, 10]. However, the ideal agent to protect against

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cartilage desiccation would be easily applied, requires infrequent refreshing, does not interfere with the surgical procedure and is cost-effective.

This study was undertaken to confirm the chondrotoxicity of air and compare several commonly used surgical compounds and investigate their chondroprotectivity in a controlled laboratory. To our knowledge, this is the first study to investigate cost-effective and easy applicable options for the operating room to protect cartilage exposure to air.

Materials and methods

Arthrotomies were performed on five freshly harvested calf knees in an orthopaedic surgical laboratory under sterile conditions. Joint surfaces were examined to ensure that they were grossly intact. Osteochondral plugs from five bovine knees ($n = 16$ per knee) measuring 10 mm in diameter were obtained from femoral condyles using OATS (Arthrex Inc., Naples, FL) harvesters. Full-thickness cartilage sections were then obtained by sharply dividing the hyaline cartilage portion from the subchondral bone at the calcified cartilage layer. The cartilage sections were immediately stored in nutritional media composed of Dulbecco's Modified Eagle Medium/F-12 supplemented with 10 % foetal bovine serum, 1 % penicillin/streptomycin and 1 % fungizone (DMEM/F12, Invitrogen, Grand Island, NY, USA) and transported within 20 min to the basic science laboratory for further processing.

Articular surface treatment

The articular surface of the cartilage sections from five animals from a slaughter house—therefore, no IRB approval was necessary—were randomly divided into four groups (each group consisted of 5 plugs) and consisted of a (1) control group (air exposure), (2) Seprafilm (Genzyme, Boston, MA, USA), (3) surgical grade lubricant jelly (MediChoice, Mechanicsville, VA, USA) or (4) LR (Baxter, Deerfield, Illinois, USA). The time between the animal killing and the delivery remained under 2 h. The cartilage plugs were taken out of the nutritional media and placed in a well plate. The plugs were then treated with each agent once at the beginning of the experiment and then exposed to air at ambient room temperature for 0, 30, 60, 120 min. Surgical lube and Seprafilm, which were dipped in saline before applying to the cartilage plug, were applied for a few seconds and then wiped off with a sterile swab at time point 0. Rewetting of the LR occurred every 20 min to compare with a previous study [13]; no additional treatments were reapplied for Seprafilm and surgical grade lube. A control, the fourth group, was used for comparison in

which no agent was used. Immediately after treatment and at the subsequent time points, the plugs ($n_{\text{total}} = 80$) were immersed in DMEM/F12 and incubated at 37 °C in 5 % CO₂ for 48 h, and subsequently assessed for chondrocyte viability.

Chondrocyte viability

A cryomicrotome (Leica, Allendale, NJ, USA), set at room temperature was utilized to obtain 50- μm -thick slices from the middle third of each articular plug, oriented perpendicularly to the articular surface. Next, chondrocyte viability was quantified with a Live/Dead viability/cytotoxicity kit (Molecular Probes, West Eugene, OR) following manufacturer's specifications, which has been shown to assess cell viability effectively in dense human connective tissue, including cartilage [6]. Briefly, cartilage slices were stained with calcein-AM and ethidium homodimer-1 with the recommended concentrations for 45 min. Fluorescent images were obtained with an Olympus IX70 fluorescent microscope equipped with a SPOT digital camera and image processing software (Diagnostic Instruments, Sterling Heights, MI). The results are represented as the percentage of living cells in the area of 500 μm of depth as described previously [13].

Statistical analysis

StataSE software (StataCorp, College Station, TX) was used to perform a two-way analysis of variance on the data set. Tukey's HSD post hoc test of the means of each group was then used to determine statistical significance. The independent factors were treatment duration and treatment modality. Statistical significance was determined at the 0.05 level. G*Power software (Heinrich-Heine-University, Düsseldorf, Germany) was used to perform a post hoc power analysis for each group at each experimental time point. The post hoc power analysis was performed on the difference between the two independent sample means with a two-tailed distribution and a 0.05 level of significance. A total of 80 cartilage plugs were included in this study, with 5 cartilage plugs included in each experimental condition (4 conditions) at each time point (4 time points), totalling 16 experimental sample groups. This approach ensured a statistical power of at least 80 % for all tested sample groups.

Results

Chondrocyte viability demonstrated a significant correlation between duration of exposure and depth of chondrocyte death in each group ($p < 0.001$).

In the control group, the viability of articular cartilage decreased steadily within the first 500 μm , when exposed to room air from $82.0 \pm 5.7\%$ (range 74.8–88.2 %) at time point 0 to $33.0 \pm 9.1\%$ (range 19.8–39.2 %) at 120 min ($p < 0.0001$). Similarly, in all other treatment groups, viability decreased with increasing time ($p < 0.001$) (Fig. 1a, b, c, d).

At time point 0, treatment with LR appeared to have the most chondroprotective potential of all 4 groups (Fig. 2). No significant differences were seen when the control ($82.0 \pm 5.7\%$, range 74.8–88.2 %) was compared with surgical lube ($84.0 \pm 1.7\%$, range 82.0–85.8 %) (n.s.) (Table 1).

Exposure for 30 min to ambient room air resulted in decreased viability in all treatment groups. Surgical grade lube as well as the lactated ringer group had the highest percentage of living cartilage cells and were not statistically different from each other, i.e. $76.0 \pm 2.7\%$ (range 74.0–79.8 %) versus $81.0 \pm 1.2\%$ (range 79.6–82.4 %) (n.s.) (Table 1).

After 1 h of air exposure, the group initially treated with surgical grade lube demonstrated the highest chondroprotective effects in comparison with Septrafilm, LR and the control, i.e. $70.0 \pm 6.8\%$ (range 64.6–79.8 %) versus $45.0 \pm 16.3\%$ (range 25.6–60.0 %), $45.0 \pm 2.0\%$ (range

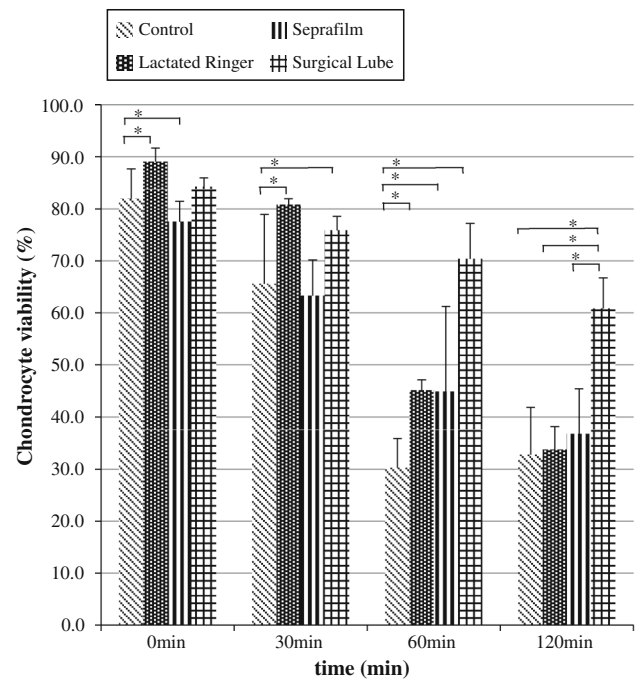


Fig. 2 Chondrocyte Viability in correlation to exposure time. Bar chart showing that exposure to surgical lube had the greatest chondroprotectivity in the superficial zone of the articular cartilage. The amount of chondrotoxicity was time-dependent. There was no cell death beyond 500 μm

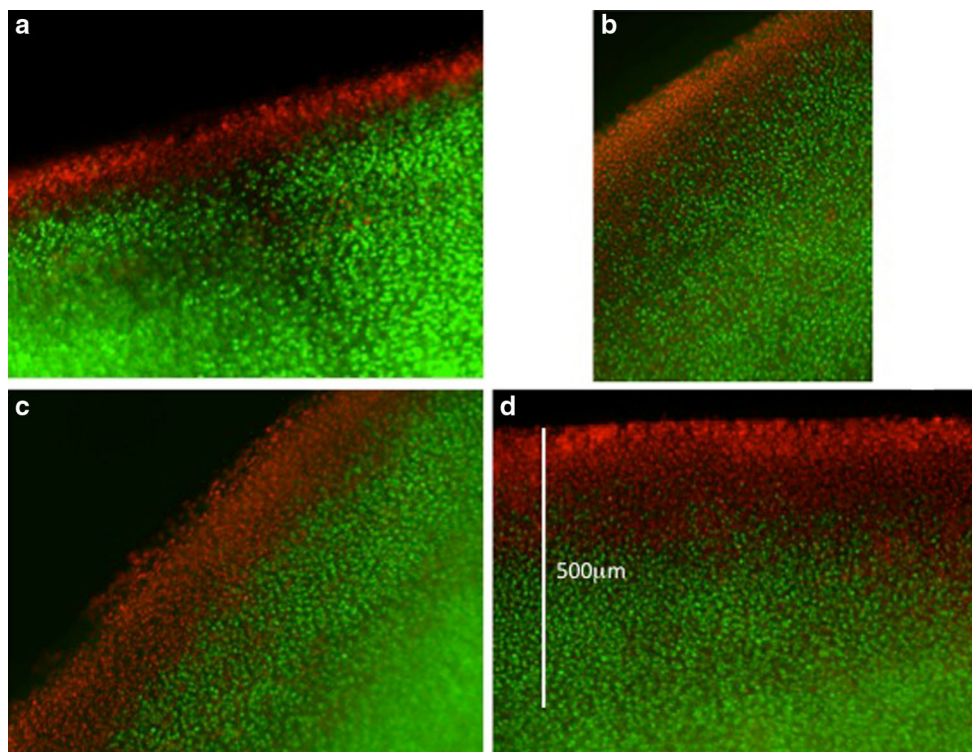


Fig. 1 Photomicrographical measurement of chondrocyte viability. Photomicrographs of full-thickness cartilage specimen analyzing chondrocyte viability using Live/Dead staining after exposure to surgical lube for **a** 0 min, **b** 30 min, **c** 60 min and **d** 120 min. The

surface of the osteochondral plugs is at the *top* of the image. Viable chondrocytes are stained with calcein-AM (*green*) and dead chondrocytes with ethidium homodime-1 (*red*)

Table 1 Viability of articular cartilage measured in percentage of living chondrocyte cells within the first 500 μm

	0 min	30 min	60 min	120 min
Air	82.0 \pm 5.7	66.0 \pm 13.3	30.0 \pm 5.7	33.0 \pm 9.1
Lactated ringer	89.0 \pm 2.6	81.0 \pm 1.2	45.0 \pm 2.0	33.8 \pm 4.4
Septrafilm	78.0 \pm 3.9	63.0 \pm 6.9	45.0 \pm 16.3	37.0 \pm 8.7
Surgical grade lube	84.2 \pm 1.7	76.0 \pm 2.7	70.0 \pm 6.8	61.0 \pm 5.9

42.4–47.0 %); 30.0 \pm 5.7 % (range 24.4–35.6 %) (all $p < 0.0001$) (Table 1).

After 2 h, surgical lube (61.0 \pm 5.9 %, range 53.2–67.0 %) better maintained chondrocyte viability in comparison with LR (33.8 \pm 4.4 %, range 29.0–37.8 %), Septrafilm (37.0 \pm 8.7 %, range 25.8–44.4 %) and control (33.0 \pm 9.1 %, range 19.8–39.2 %) (all $p < 0.0001$) (Table 1).

Discussion

The most important finding of this present study was that with simple measures, we were able to reduce the amount of chondrocyte death by applying a thin layer of surgical lubricant to the native cartilage. Cartilage is an aneural and avascular structure with a very limited capacity to heal, which emphasizes the importance of minimizing iatrogenic damage from desiccation during open procedures. Intra-articular reconstruction after trauma and cartilage regenerative procedures often require an extended operative time, thus leaving healthy cartilage exposed to room air. In shorter knee, hip or shoulder procedures of 30 min or less, LR or no treatment appears to be sufficient. For exposures exceeding 60 min, surgical lubricant demonstrated the highest protective potential.

It has been shown that room air for an extended period of time damages the superficial layer of cartilage [8, 13]. Similarly, Han et al. [5] have found chondrocyte necrosis in knees of rabbits after exposure to air with increasing toxicity from 30 min to 2 h in the superficial one-third of the surface. Mitchell et al. [8] and Bloebaum et al. [1] have confirmed the findings of other studies that periodic rewetting of exposed cartilage with LR solution decreases the deleterious effect of room air. However, periodic rewetting every 5, 10 or 20 min may be overlooked during long orthopaedic procedures.

Pun et al. [13], have reported on the chondroprotective ability of periodic rewetting with LR when analysing the first 500 μm of human cartilage. The viability increased from 20 % when no LR was applied to 60 % when the cartilage surfaces were rewetted every 10 min after 2 h.

Similarly, we have found that the viability in bovine cartilage in the superficial 500 μm decreased to 33 % at 120 min when no protective measures were taken. However, when we treated the native cartilage with a thin layer of surgical lubricant, the viability of chondrocytes increased to 61 %. This might be useful for long and complex procedures, as the surgical lubricant is employed only once, then left in place without further intervention necessary. In contrast to Pun et al. [13] findings, the experiment groups in this study treated with LR resulted in only 34 % viable cells. This difference may be due to decreased frequency with rewetting only every 20 min, rather than every 10 min, which might be a more accurate reflection of clinical reality.

The application of Septrafilm, consisting mainly of hyaluronate and carboxymethylcellulose, appeared not to be chondroprotective to the surface cartilage. It decreased the viability to 78 % at time point zero and further diminished to 37 % in a surface depth of 500 μm after 2 h, similar to LR. This is different than findings by Bloebaum et al. [1]. Who presented data that supported the use of hyaluronic acid for the protection of cartilage tissue. It is possible that carboxymethylcellulose acts as a desiccant and thus, without rewetting it with LR, leads to the observed increase in the rate of chondrocyte death. In a study by Lui et al. [7], different concentrations of hyaluronic acid has been found to be chondroprotective against the chondrotoxic effect of supraphysiologic temperatures and concomitant application of 0.25 and 0.5 % Bupivacaine. However, even though using the same method to detect viability of chondrocytes, their setup was different. In comparison with their study, we did not use a liquid chondrocyte cell media in combination with different solutions, but studied cartilage viability in osteochondral explants.

The exact mechanism of action of the precipitating factors still remains to be determined. However, the surface cartilage appears to play a crucial part in preventing the start of the osteoarthritis cascade [4]. It has been shown that superficial cartilage zone protein, which is a proteoglycan that is produced by chondrocytes in the superficial layer, is one of the key factors in the protection against pathophysiologic influences [14, 15]. This protein has been found to be distinct from lubricin [17, 18], another protective surface protein [14]. Thus, it appears to be of great importance to prevent damage to the surface layer. We did not measure cartilage zone protein but used the surface cartilage cell death as an indicator as described previously [3, 4, 8, 11, 12, 19].

A major limitation of this current study is the use of non-human cartilage. A bovine osteochondral explant in vitro model was used because it allows histological evaluation of the superficial layer of fresh, normal

cartilage. The supply of similarly fresh and normal human cartilage is extremely limited: discarded tissue after total knee replacement is mostly affected by degenerative changes, and allograft osteochondral tissue has already been exposed to prolonged storage conditions with the development of chondrocyte death. Prior studies have found that bovine articular chondrocytes respond similar to human articular chondrocytes after exposure to bupivacaine and thermal stress [2]. Although further studies in human articular cartilage are necessary, findings in this study could prevail to be clinically relevant. The small number of specimens ($n = 5$) is another limitation; however, each experiment was repeated 5 times per specimen. Additional studies would be desirable to confirm the data of this study in human cartilage tissue in vivo and in vitro. Furthermore, the live/dead staining technique utilized is based on specific cell metabolic products commonly associated with cell death. However, Speer et al. [16] have proposed that some of these findings may be reversible within 6 weeks, a time period not conducive to in vitro experiments such as the one found in the present study.

Conclusion

Previous studies have demonstrated the negative effects of desiccation on articular cartilage viability. This study found that surgical grade lubricant, which is relatively low cost, adheres well to articular surfaces and required only a one-time application, is effective in protecting articular cartilage from desiccation for up to 2 h. Our study indicates that protecting exposed articular cartilage surfaces with surgical lubricant, for orthopaedic procedures lasting more than 1-h, decreases articular surface chondrocyte death and may lead to improved cartilage functional outcomes postoperatively.

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