Periodic rewetting enhances the viability of chondrocytes in human articular cartilage exposed to air

S. Y. Pun, M. S. Teng, H. T. Kim
From the University of California, San Francisco, USA

Desiccation of articular cartilage during surgery is often unavoidable and may result in the death of chondrocytes, with subsequent joint degeneration. This study was undertaken to determine the extent of chondrocyte death caused by exposure to air and to ascertain whether regular rewetting of cartilage could decrease cell death.

Macroscopically normal human cartilage was exposed to air for 0, 30, 60 or 120 minutes. Selected samples were wetted in lactated Ringer’s solution for ten seconds every ten or 20 minutes. The viability of chondrocytes was measured after three days by Live/Dead staining.

Chondrocyte death correlated with the length of exposure to air and the depth of the cartilage. Drying for 120 minutes caused extensive cell death mainly in the superficial 500 µm of cartilage. Rewetting every ten or 20 minutes significantly decreased cell death.

The superficial zone is most susceptible to desiccation. Loss of superficial chondrocytes likely decreases the production of essential lubricating glycoproteins and contributes to subsequent degeneration. Frequent wetting of cartilage during arthrotomy is therefore essential.

During arthrotomy, articular cartilage may suffer prolonged exposure to air. The resulting dehydration can precipitate chondrocyte death which in turn may lead to degenerative change.

In spite of its obvious importance, little is known about cell death induced by the exposure of cartilage to air. Published data are available only for rabbit cartilage which has considerable biochemical and morphological differences from human cartilage. The latter is much thicker in most joints and this alone is likely to alter the reaction of chondrocytes to desiccation.

Although surgeons are trained to minimise desiccation by irrigating articular surfaces, the magnitude of damage to exposed human cartilage is unknown. It is also not clear how frequently human cartilage must be rewetted to prevent injury. Because articular cartilage has very limited capacity for healing, it is important to develop techniques which minimise unnecessary cell death. Our study was undertaken to determine the extent of cell death caused by exposure of human articular cartilage to air and to ascertain whether regular rewetting could decrease the damage.

Materials and Methods
Fresh osteochondral specimens were obtained from the femoral condyles of six human knees from donors aged between 17 years and 80 years, either after above-knee amputation, or within 24 hours of death. All specimens were screened for gross evidence of degeneration or injury to the cartilage. The tissue collection was approved by our Institutional Review Board.

Full-thickness samples of cartilage from the weight-bearing region were taken using a 4 mm dermal biopsy punch after 0, 30, 60 and 120 minutes of exposure to room air at ambient room temperature. Three biopsy samples from each time point were maintained under standard conditions in Dulbecco’s modified eagle medium (UCSF Cell Culture Facility, San Francisco, California), supplemented by 10% fetal bovine serum. After three days, the samples were analysed for the number and distribution of living and dead cells. Three independent experiments using three femora were performed.

In order to study the effect of rewetting, osteochondral specimens were exposed to air for 120 minutes, during which time each was submerged in lactated Ringer’s solution (Baxter, Deerfield, Illinois) for ten seconds at intervals of either ten or 20 minutes. Three biopsy samples obtained from each specimen were maintained under standard conditions as before, and assayed for cell viability after three days. Three independent experiments using three femora were performed.
A vibratory microtome (Vibratome, St. Louis, Missouri) was used to obtain tissue for analysis of cell viability. Cylindrical samples of cartilage were mounted on plastic blocks and slices 50 µm thick orientated perpendicular to the articular surface were taken from the middle third of each. The viability of chondrocytes was quantified by staining with calcein-AM and ethidium homodimer-1 (EthD-1) in the Live/Dead Viability/Cytotoxicity kit (Molecular Probes, West Eugene, Oregon), which has been shown to assay cell viability effectively in dense human connective tissue, including cartilage. Live cells were stained with calcein-AM and dead cells with EthD-1. Concentrations of dye were modified from the manufacturer’s protocol to optimise the sensitivity and specificity of the assay.

Fluorescence images were captured by an Axiocam digital camera (Carl Zeiss, Thornwood, New York) at a resolution of 1 megapixel. For each sample, Vibratome sections were screened for processing artefacts and a representative image selected for analysis. The fields included the full thickness of articular cartilage extending out to 2.0 mm from the centre. The area for analysis was 1.0 mm deep from the articular surface and 2.0 mm wide. Semi-automated data collection and analysis were carried out using Adobe Photoshop (Adobe Systems Inc., San Jose, California) and a public domain Java image processing program (ImageJ; National Institutes of Health, Bethesda, Maryland). The results were represented as the percentage of calcein-AM-positive cells, calculated by dividing the number of calcein-AM-positive cells by the total number of cells in the area of analysis.

For each experiment and for the pooled data this calculation was performed with an analysis of variance (ANOVA) using Tukey’s correction for multiple post-hoc comparisons by microcomputer (SPSS Inc., Chicago, Illinois). Statistical significance was defined as \( p < 0.05 \).
Results
The effect of exposure was grossly apparent. After as little
as 30 minutes, the cartilage lost its normal sheen. With
longer exposure it obtained a tan colour, the superficial
layer became tough and rubbery and the thickness
decreased. However, after three days in cell-culture condi-
tions, the samples appeared to be rehydrated and regained
normal tissue thickness.

In order to determine the extent of chondrocyte death,
the osteochondral specimens were exposed to air for up to
120 minutes and processed after three days for Live/Dead
analysis. The pattern of cell death shows an obvious area of
death chondrocytes extending for a fairly uniform distance
from the superficial surface (Fig. 1). Univariate ANOVA
showed that the duration of exposure to air and the depth
of the chondrocytes had significant effects on viability (p <
0.001 for both). Exposure to air had a more pronounced
effect on superficial chondrocytes (Fig. 2). Within the most
superficial 250 µm of articular cartilage, exposure for 30
minutes decreased viability from a mean of 81% ± 3.8% SEM
to a mean of 57% ± 6.3% SEM (p = 0.002). After one
hour, mean viability decreased to 41% ± 3.6% SEM (p <
0.001) and there were virtually no viable chondrocytes (3% ±
2.0% SEM) after two hours (p < 0.001). Within the next
250 µm of cartilage (250 µm to 500 µm), exposure to air
for two hours decreased viability to 29% ± 11.3% SEM
(p = 0.001). Exposure for 60 minutes or less did not result
in a statistically significant loss of cell viability at this depth.
Within the deeper areas (from 500 µm to 750 µm and 750 µm
to 1000 µm) exposure for any time up to two hours did not
cause significant cell death.

In order to determine whether chondrocyte death could
be decreased by intermittent rewetting, the osteochondral
specimens were exposed to air for 120 minutes, rewetted
with lactated Ringers’ solution every ten or 20 minutes,
then processed for Live/Dead analysis (Fig. 3). These speci-
mens maintained a grossly normal appearance throughout
the 120 minutes of desiccation. Rewetting every ten min-
utes consistently and significantly decreased cell death in
the superficial 500 µm of articular cartilage compared with
no rewetting (60% ± 8.8% SEM viability vs 20% ± 4.5% SEM;
p < 0.001). Rewetting every 20 minutes also decreased
cell death in the superficial 500 µm (45% ± 6.1% SEM viability
vs 20% ± 4.5% SEM viability; p < 0.001). Although
rewetting every ten minutes was significantly more effective
than every 20 minutes (p = 0.005), it was not sufficient to
maintain chondrocyte viability compared with specimens
not exposed to air (p < 0.001).

Discussion
Since articular cartilage has little, if any, ability to heal it is
important to minimise iatrogenic damage during surgery. In
complex operations, cartilage may be exposed to air for
120 minutes or longer. Our findings show the fatal con-
sequences of such exposure for articular chondrocytes and,
in spite of best intentions, it is unlikely that surgeons would
irrigate exposed cartilage often enough to avoid unneces-
sary cell death.
Intermittent rewetting during prolonged exposure is crucial for the viability of human chondrocytes. The more frequently this is done the better is their preservation. It has been shown that irrigating rabbit cartilage either every five minutes with lactated Ringers’ solution or every 30 minutes with hyaluronan solution decreases necrosis of chondrocytes due to desiccation.\(^1,2\) Clinically, the irrigation of cartilage every five minutes may be impractical, but rewetting every ten to 20 minutes with lactated Ringers’ solution is simple, applicable and effective.

Regardless of efforts to maintain hydration, the duration of exposure appears to be the most significant determinant of chondrocyte death. Exposure for 30 minutes caused less death than that for 120 minutes with rewetting every ten or 20 minutes. This implies that finishing an operation more quickly with no rewetting is less injurious to articular cartilage than a prolonged operation with intermittent rewetting.

It may be reassuring that cell death is predominantly limited to the most superficial 0.5 mm of articular cartilage, even after exposure for two hours. However, the loss of these superficial chondrocytes may have a disproportionately large effect on the degeneration of cartilage due to their unique biochemical properties. The superficial tangential zone is particularly important for lubrication. During normal cyclic loading, articular cartilage exudes a very thin fluid film to reduce friction and wear. Exudation of extracellular matrix fluid is limited to the most superficial zone.\(^5\) A key component of this fluid is superficial zone protein, a lubricating glycoprotein uniquely produced by superficial zone chondrocytes.\(^6,7\) Loss of lubricating glycoproteins is associated with damage to the cartilage matrix and joint degeneration.\(^8,9\) Preservation of superficial zone chondrocytes may therefore be particularly important in preventing degeneration.

A limitation of our study is that it could only be performed in vitro since it is ethically impossible to conduct such a study in vivo in patients. Also, whereas we focused on the short-term response to desiccation, the real issue is its long-term effects. In vivo studies on large animals with articular cartilage which has a thickness similar to that of human cartilage may provide a better picture of the long-term effects of desiccation.

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References

Bar chart showing that briefly rewetting cartilage with lactated Ringers’ solution every ten or 20 minutes markedly decreased chondrocyte death caused by air exposure for 120 minutes. Rewetting every ten minutes allowed preservation of a greater percentage of chondrocytes than did rewetting every 20 minutes (p = 0.005). However, rewetting every ten minutes did not preserve chondrocyte viability to the baseline level as seen with no air exposure at all.


