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Airflow accelerates bovine and human articular cartilage drying and chondrocyte death

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SUMMARY

Objective: Exposure of articular cartilage to static air results in changes to the extracellular matrix (ECM) and stimulates chondrocyte death, which may cause joint degeneration. However during open orthopaedic surgery, cartilage is often exposed to laminar airflow, which may exacerbate these damaging effects. We compared drying in static and moving air in terms of cartilage appearance, hydration and chondrocyte viability, and tested the ability of saline-saturated gauze to limit the detrimental effects of air exposure.

Design: Articular cartilage from bovine metatarsophalangeal joints ($N = 50$) and human femoral heads ($N = 6$) was exposed for 90 min to (1) static air (2) airflow (up to 0.34 m/s), or (3) airflow (0.18 m/s), covered with gauze. Following air exposure, cartilage was also rehydrated (0.9% saline; 120 min) to determine the reversibility of drying effects. The influence of airflow was assessed by studying macroscopic appearance, and quantifying superficial zone (SZ) chondrocyte viability and cartilage hydration.

Results: Airflow caused advanced changes to cartilage appearance, accelerated chondrocyte death, and increased dehydration compared to static air. These effects were prevented if cartilage was covered by saline-saturated gauze. Cartilage rehydration reversed macroscopic changes associated with drying but the chondrocyte death was not altered. Chondrocytes at the cut edge of cartilage were more sensitive to drying compared to cells distant from the edge.

Conclusions: Airflow significantly increased articular cartilage dehydration and chondrocyte death compared to static air. As laminar airflow is routinely utilised in operating theatres, it is essential that articular cartilage is kept wet via irrigation or by covering with saline-saturated gauze to prevent chondrocyte death.

Introduction

Exposure of articular cartilage to air results in tissue drying, chondrocyte death, and alterations to the extracellular matrix (ECM)[1–4]. A range of changes have been reported, including an increase in articular surface roughness observed *via* profilometry[5], glycosaminoglycan depletion measured by staining with cationic dyes[3], and chondrocyte trauma and death, identified by transmission electron[1–3] and confocal laser scanning microscopy (CLSM)[4]. While the exact mechanism and permanence of the changes remain unknown, the potential dangers of cartilage drying during surgery have been highlighted as it may contribute to post-operative joint degeneration[4].

Cell death has been shown to correlate with the duration of drying and to be progressive, starting with cells in the superficial zone (SZ) of cartilage[1–4]. Previous studies in leporine cartilage have suggested that features associated with cell death (nuclear homogenization, cell membrane rupture, and cellular retraction from the matrix) are localized to cells in the SZ after brief air exposure ($\leq$30 s) but extend into deeper zones with prolonged ($\geq$60 min) drying[1–3]. Similarly, in human cartilage, the percentage of chondrocyte death in the SZ exceeded that in deeper zones after 30, 60, and 120 min of drying[4].

Articular cartilage is commonly exposed to air during surgical procedures (e.g., cartilage repair techniques, joint preservation procedures, and intra-articular fracture surgery). Drying during surgery can be minimised by repeated irrigation; however, the frequency is vital[1,4]. Mitchell and Shepard[1] first reported that

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irrigation with Ringer's lactate solution at 5 min intervals prevented cell death. Additionally, Pun and colleagues[4] observed a significant reduction in cell death in human cartilage irrigated every 10 or 20 min and further noted that more frequent irrigation conferred greater protection. A thin layer of surgical lubricant applied as a single treatment to articular cartilage has also been shown to reduce chondrocyte death associated with drying[6].

Although some effects of drying and air exposure have been examined experimentally[1–7], this was routinely performed in 'room air'[2,4,6]. Most modern orthopaedic operating theatres are routinely equipped with specialised laminar airflow ventilation systems, with the airflow exceeding 0.2 m/s at the operating table level[8,9]. Therefore, the hazardous effects of air exposure on cartilage that have been reported[2,4,6] may not represent actual 'in-theatre' conditions, as the effect of airflow does not appear to have been previously considered.

To address this, the aim of this study was to assess the effect of increased airflow on cartilage drying. Our hypothesis was that the effects of drying on articular cartilage and chondrocytes would be exacerbated by exposure to airflow. We compared the appearance of cartilage, chondrocyte death, and water loss between control tissue (dried in static air), and experimental tissue dried in airflow or covered using a saline-soaked gauze. Additionally, we assessed the degree of chondrocyte death in response to varying airflow rates and durations.

Materials & methods

Tissue procurement and preparation

Bovine metatarsophalangeal joints from fifty animals (36 ± 2 month-old) were sourced from a local abattoir and used within 24 h. Human articular cartilage was obtained with Ethical Permission (Tissue Governance, NHS Lothian) and consent from six patients who underwent hip hemiarthroplasty for a fractured neck of femur. Human articular cartilage – comprising the entire femoral head of the hip joint – was confirmed to be non-degenerate from clinical evaluation and radiological records. The femoral head was then immediately placed in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Paisley, UK) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco, Paisley, UK) and used within 12 h of surgery. The median age of the human donors was 87 yrs (range 62–89 yrs). Bovine and human joints were visually assessed and only cartilage with a smooth and shiny (hyaline-like) gross appearance without any damage or fibrillation was used.

Open bovine joints with cartilage exposed were used when investigating gross changes in cartilage appearance and the effect of airflow on the percentage cell death (PCD). Alternatively, osteochondral explants, consisting of full thickness cartilage and approx. 1–2 mm of subchondral bone, were used for assessments of bovine cartilage water content and for all measurements on human cartilage (PCD, water content). Osteochondral explants were trimmed to approx. 5 × 5 mm with subchondral bone retained in order to limit deformation of the cartilage surface and reflect the *in situ* response to drying. Explants were sampled using a number-24 scalpel blade and trimmed using two blades (24 blade) bound together to achieve parallel cuts to give reproducible explants.

Of the fifty bovine joints, twenty were used for assessing the effect of airflow rate on PCD, in eight explants harvested from each joint. A further eighteen joints (minimum of four explants per joint) were used to compare the effect of the three main treatment groups on PCD. Water loss was assessed in twenty-five osteochondral explants taken from a further six joints. Eighteen osteochondral explants from the final six joints were used to determine the effect of rehydration on drying induced cell death (see Supplementary Table 1).

Osteochondral explants ($n = 130$) were harvested from six human femoral heads (see Supplementary Table 1). Seventy-nine of these (from three joints) were used to compare the effect of the three main groups on PCD. Water loss in these treatment groups was assessed in the remaining fifty-one osteochondral explants (from four femoral heads).

Drying protocol

Two treatment groups were used throughout this study: (1) the 'airflow' group, exposed to an airflow rate of 0.18 m/s; and (2) the 'covered' group, exposed to air at an airflow rate of 0.18 m/s but covered with sterile surgical gauze saturated in 0.9% saline solution. The control ('static' air group) was exposed to static air only. As the relationship between air exposure and chondrocyte death is well established[1–7], no unexposed controls were used. Experiments were carried out in a microbiological safety cabinet (Model Bio2+, Envair, Rossendale, UK). In order to remove residual synovial fluid prior to experimentation, joints and osteochondral explants were gently rinsed with DMEM or 0.9% saline (Baxter Healthcare, Newbury, UK).

Additionally, a subsample of bovine joints were dried in a horizontal laminar flow cabinet (Model HFC 120, Rayair, Bolton, UK) at airflows up to 0.34 m/s for 50 min. All airflows reported were taken from hood output displays and confirmed using an anemometer, and experiments were performed at 21°C. Bovine joints and osteochondral explants were rehydrated in 0.9% saline for 120 min following drying in order to assess the effect of rehydration on cartilage appearance and chondrocyte viability.

Assessment of macroscopic changes in cartilage

Bovine joints were visually assessed for changes in their appearance throughout treatment in each of the groups. A progressive and characteristic pattern of cartilage discolouration was identified in drying bovine joints. These changes were observed at varying levels of progression in all joint surfaces exposed to air. In order to compare this qualitative data, these changes were used to devise a descriptive table (see Table I), which was used to retrospectively score the extent of drying in a subsample of joints.

Assessment of chondrocyte viability

In situ superficial chondrocytes were fluorescently-labelled immediately following the experimental procedure by incubating explants in DMEM supplemented with Cell Tracker™ Green 5-chloromethylfluorescein diacetate (CMFDA) and propidium iodide (PI) (Invitrogen, Paisley, UK) as described[10]. For the purposes of this study, the SZ was defined as the superficial 100 μm of tissue (c.20% of the tissue thickness). To optimize images for chondrocytes within human cartilage, the CMFDA concentration was maintained at 10 μM, however the PI concentration was increased to 20 μM and labelling was performed with gentle agitation for 2 h at 21°C. These dyes labelled the cytoplasm of living cells and nuclei of dead cells respectively and are well established in cartilage and connective tissue research[11]. Fluorescently-labelled chondrocytes were imaged using a Zeiss Axioskop LSM510 (Carl Zeiss, Welwyn Garden City, UK) with a low power (x10 dry, NA = 0.3) objective lens. A series of axial images of *in situ* superficial chondrocytes were acquired at 10 μm intervals typically to a depth of about 100 μm, so as to produce a three-dimensional projection of explants[10].

The PCD was quantified in a three-dimensional region of interest (ROI) (XYZ dimensions 700 μm × 921 μm × 100 μm) using