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Effects of damage in the articular surface on the cartilage response to injurious compression in vitro

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Abstract

Macroscopic structural damage to the cartilage articular surface can occur due to slicing in surgery, cracking in mechanical trauma, or fibrillation in early stage osteoarthrosis. These alterations may render cartilage matrix and chondrocytes susceptible to subsequent mechanical injury and contribute to progression of degenerative disease. To examine this hypothesis, single 300 μm deep vertical slices were introduced across a diameter of the articular surface of osteochondral explant disks on day 6 after dissection. Then a single uniaxial unconfined ramp compression at 7×10^{-5} or $7 \times 10^{-2}\,s^{-1}$ strain rate to a peak stress of 3.5 or 14 MPa was applied on day 13 during which mechanical behavior was monitored. Effects of slices alone and together with compression were measured in terms of explant swelling and cell viability on days 10 and 17. Slicing alone induced tissue swelling without significant cell death, while compression alone induced cell death without significant tissue swelling. Under low strain rate loading, no differences in the response to injurious compression were found between sliced and unsliced explants. Under high strain rate loading, slicing rendered cartilage more easily compressible and appeared to slightly reduce compression-induced cell and matrix injury. Findings highlight microphysical factors important to cartilage mechanical injury, and suggest ways that macroscopic structural damage may accelerate or, in certain cases, possibly slow the progression of cartilage degeneration.

Keywords: Cartilage; Inquiry; Mechanical compression; Viability; Slice; Defects

1. Introduction

The progression of osteoarthrosis (OA) characteristically involves macroscopic structural changes to articular cartilage (Buckwalter and Mankin, 1998). Such changes can arise in various contexts. Superficial cracking due to mechanical trauma (Jeffrey et al., 1995; Ewers et al., 2001; Morel and Quinn, 2004a) and deliberate or accidental slicing during surgery (Brittberg et al., 1994; Gautier et al., 2002; Hunziker, 1999) are associated with the initiation of OA. Superficial fibrillation in early phases of OA involves macroscopic changes in cartilage structure relevant to disease progression (Buckwalter and Mankin, 1998). These structural changes can alter the tissue mechanical response to joint loading and may thereby contribute to the mediation of disease progression by mechanical loading. Study of the cartilage response to mechanical loading in the context of macroscopic structural damage is therefore relevant to better understanding of factors contributing to the initiation and progression of OA (Heinegard et al., 1998).

Macroscopic structural changes in the cartilage articular surface may alter tissue mechanical behavior and affect microphysical stimuli conveyed to chondrocytes during tissue loading. Previous studies have shown that cartilage slicing induces swelling of the extracellular matrix (Setton et al., 1998), which can lead to alterations of tissue mechanical properties (Armstrong and Mow, 1982; Eisenberg and Grodzinsky, 1985) and

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consequently to changes in the matrix mechanical response to loading. Longer-term responses to surgical manipulation of cartilage may involve focal matrix degeneration due to localized loss of cell viability (Hunziker and Quinn, 2003; Redman et al., 2004). Similarly, cell death near to cracks resulting from high strain rate cartilage injury (Jeffrey et al., 1995; Ewers et al., 2001; Morel and Quinn, 2004a) likely leads to alterations in matrix mechanical competence due to matrix remodelling mediated in part by the remaining viable cells (Quinn et al., 1998; Lin et al., 2004). Focal changes in matrix mechanical properties together with alterations in the tissue geometry itself due to slicing, cracks, or fibrillation may introduce important changes in mechanical events occurring within relatively normal cartilage nearby during joint loading. These modifications might thereby perturb the cell biological response to physiological tissue compression (Kurz et al., 2001) and alter remodelling pathways contributing to degenerative disease. The cartilage response to subsequent injurious compression may also be affected, such that nonphysiological mechanical loading has more deleterious effects than for structurally normal cartilage.

We developed an in vitro model to study effects of damage in the articular surface on the subsequent cartilage response to injurious compression. Our goals were to examine the effects of a geometrically well-defined surface lesion (serving as a model for surgical slices, tissue cracks, or mild fibrillation) on the cartilage mechanical response to injurious compression and acute manifestations of cell and matrix injury relevant to the initiation and progression of OA. Vertical slices of 300 μm depth were introduced across a diameter of the articular surface of cylindrical osteochondral explants before application of a well-characterized injurious compression protocol. Mechanical behavior was monitored during compression and acute effects of slices alone and together with compression were measured in terms of explant swelling and cell viability.

2. Methods

4 mm diameter osteochondral cores were drilled from fresh 18-month old bovine humeral heads under saline irrigation. The full-thickness cartilage layer was trimmed to 2.7 mm diameter and the bone to 1 mm thickness as previously described (Morel and Quinn, 2004a). Explants were incubated (at 37 °C and 5% CO_2) for up to 17 days (Fig. 1a) in DMEM (Oxoid AG, Basel, Switzerland) containing 1 g/L D-Glucose, 3.7 g/L $NaHCO_3$, 1 g/L *N*-acetyl-lanalyl-L-glutamine, and supplemented with 0.1 mM non-essential amino acids (Oxoid), 0.4 mM L-Proline (Sigma, Buchs, Switzerland), 10 mg/mL streptomycin sulfate, 10,000 units/mL penicillin G sodium, 25 μg/mL amphotericin B (Gibco BRL, Basel,

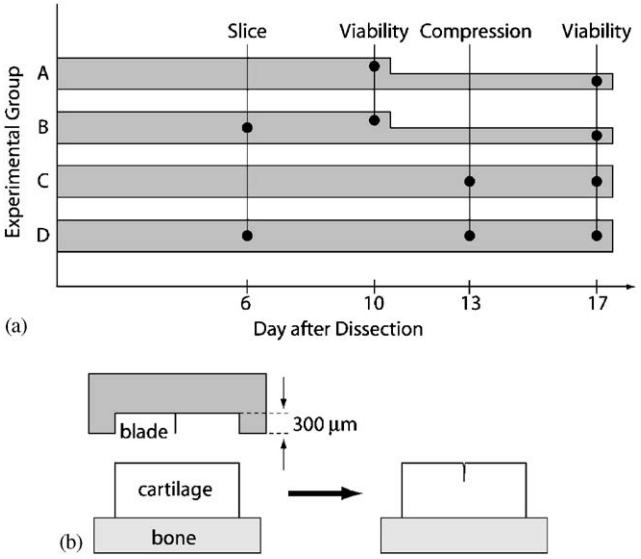

Fig. 1. (a) Experimental timeline and distribution of explants into experimental groups. Group A: unsliced and uncompressed controls; Group B: sliced only; Group C: compressed only; Group D: sliced and compressed. (b) Schematic of tool for introduction of 300 μm deep slices in the articular surface of osteochondral explants.

Switzerland), and 10% (v/v) fetal bovine serum (Sigma), with daily changes of 1 mL media per explant. Spent media was assayed for proteoglycan release from explants by the dimethylmethylene blue method (Farndale et al., 1982). After 6 days, half of the explants received a single 300 μm deep slice, introduced vertically across a diameter of the articular surface using a razor blade mounted in a custom-made tool (Fig. 1b). This slice depth was chosen because it was large enough that it could be easily and reliably introduced, yet remained largely superficial and was similar in size to previously observed compression-induced cracks (Morel and Quinn, 2004a).

On day 13, explant geometry was measured using a microscope (NCL 150, Nikon AG, Egg, Switzerland) with reticulated eyepiece. Cartilage thickness (articular surface to tidemark) was measured and used as the reference for strain during compression which was applied on the same day. Stress was defined relative to cartilage surface area at dissection. Explants were compressed axially without radial confinement between a steel loading post on the articular surface, and a steel and plexiglass support chamber. A displacement actuator (PM500-1A, Newport Instruments, Schlieren, Switzerland) raised the support chamber to push the explant against the loading post which was attached to a load cell (Model 31, Sensotec, Schaffhausen, Switzerland). The actuator and load cell were fixed in an aluminum and stainless steel frame and interfaced with a microcomputer (Macintosh, Cupertino, CA) with instrumentation software (LabVIEW, Austin, TX). Compression was applied at a strain rate of $7 \times 10^{-2}\,s^{-1}$ (high strain