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Stress-vs-time signals allow the prediction of structurally catastrophic events during fracturing of immature cartilage and predetermine the biomechanical, biochemical, and structural impairment ☆

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ABSTRACT

Objective: Trauma-associated cartilage fractures occur in children and adolescents with clinically significant incidence. Several studies investigated biomechanical injury by compressive forces but the injury-related stress has not been investigated extensively. In this study, we hypothesized that the biomechanical stress occurring during compressive injury predetermines the biomechanical, biochemical, and structural consequences. We specifically investigated whether the stress-vs-time signal correlated with the injurious damage and may allow prediction of cartilage matrix fracturing.

Methods: Superficial and deeper zones disks (SZDs, DZDs; immature bovine cartilage) were biomechanically characterized, injured (50% compression, 100%/s strain-rate), and re-characterized. Correlations of the quantified functional, biochemical and histological damage with biomechanical parameters were zonally investigated.

Results: Injured SZDs exhibited decreased dynamic stiffness (by 93.04 ± 1.72%), unresolvable equilibrium moduli, structural damage (2.0 ± 0.5 on a 5-point-damage-scale), and 1.78-fold increased sGAG loss. DZDs remained intact. Measured stress-vs-time-curves during injury displayed 4 distinct shapes, which correlated with histological damage ($p < 0.001$), loss of dynamic stiffness and sGAG ($p < 0.05$). Damage prediction in a blinded experiment using stress-vs-time grades was 100%-correct and sensitive to differentiate single/complex matrix disruptions. Correlations of the dissipated energy and maximum stress rise with the extent of biomechanical and biochemical damage reached significance when SZDs and DZDs were analyzed as zonal composites but not separately.

Conclusions: The biomechanical stress that occurs during compressive injury predetermines the biomechanical, biochemical, and structural consequences and, thus, the structural and functional damage during cartilage fracturing. A novel biomechanical method based on the interpretation of compressive yielding allows the accurate prediction of the extent of structural damage.

1. Introduction

Articular cartilage lesions of the adult knee joint are common (Hjelle et al., 2002). In children and adolescents, the incidence of articular cartilage lesions varies depending on the cause and time of clinical presentation and the patient age. However, cartilage

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lesions in children and adolescents always occur with clinically significant incidence (Lauterburg and Segantini, 1994; Smith et al., 2005; Tallón-López et al., 2006; Stanitski et al., 1993; Sarpel et al., 2007; Stanitski, 1998; Stanitski and Paletta, 1998; Ure et al., 1992; Oeppen et al., 2004) and are, in some studies, considered the most common type of defect after trauma (Lauterburg and Segantini, 1994; Oeppen et al., 2004). Three studies reported the use of autologous chondrocyte implantation in the adolescent population (Mithofer et al., 2005; Micheli et al., 2006; Macmull, 2011) illustrating the increasing awareness of the surgical community on the clinical significance of cartilage lesions in the skeletally immature knee joint.

Macroscopically, four types of traumatic cartilage lesions are classified (Bauer and Jackson, 1988). Their morphology is associated with different mechanisms of trauma such as shear or blunt impact (Bauer and Jackson, 1988). For example, impact trauma leads to a stellate fracture of the cartilage (Bauer and Jackson, 1988) and, on tissue levels, to fissuring (Ewers et al., 2001; Rolauffs et al., 2010), cell death (Ewers et al., 2001; Loening et al., 2000), and impaired collagen integrity (Rolauffs et al., 2010; Chen et al., 2003; Repo and Finlay, 1977). In both immature and mature cartilages, traumatic lesions begin with damage to the articular surface as clinical and basic science studies demonstrated (Bauer and Jackson, 1988; Ewers et al., 2001; Rolauffs et al., 2010; Loening et al., 2000; Chen et al., 2003; Repo and Finlay, 1977). That the damage is localized to the articular surface can be explained by the presence of depth-dependent variations in the structural (Rolauffs et al., 2008, 2010; Hunziker, 1992), biochemical (Rolauffs et al., 2010; Venn and Maroudas, 1977) and biomechanical (Rolauffs et al., 2010; Schinagl et al., 1997) properties.

Several previous studies investigated the biomechanical properties of articular cartilage (Buckley et al., 2010; Buckley et al., 2008; Chen et al., 2001a,b; Grodzinsky, 1983; Klein et al., 2007) and the consequences of injury by compressive forces (Rolauffs et al., 2010; Sui et al., 2009; DiMicco et al., 2004; Kurz et al., 2005; Lee et al., 2005; Morel and Quinn, 2004; Quinn et al., 2001). However, to the best of our knowledge, the biomechanical stress (force per area) that occurs *during* injurious compression has not been investigated extensively (Rolauffs et al., 2010; Kurz et al., 2005). For example, studies examined the peak stress and the strain rate as parameters defining injury (Morel and Quinn, 2004; Patwari et al., 2007; Kurz et al., 2001) but few attempts have been made to specifically investigate the biomechanical environment *during* an impact injurious compression (Flachsmann et al., 2001). Consequently, our knowledge of the stress-vs-time profile that occurs during injurious compression and specifically the interconnections of the stress with the extent of damage are still limited. Such insight, however, is relevant for understanding the initial events during injury and, ultimately, for the development of articular cartilage lesions and posttraumatic osteoarthritis.

In this study, we investigated the biomechanical stress that occurs during injurious compression. Because impact trauma causes a specific stellate cartilage fracture (Bauer and Jackson, 1988), we hypothesized that the rise and time-course of stress during compressive injury largely determine the extent of the damage. Thus, we investigated whether the stress-vs-time signal that was recorded during injurious compression correlated with the biochemical, biomechanical and structural cartilage damage. Secondly, we investigated whether it was possible to accurately predict the extent of damage by utilizing the stress-vs-time signal in a blinded experimental approach. Finally, because damage to immature cartilage does not progress beyond the superficial zone (Rolauffs et al., 2010), we asked whether cartilage compressive properties exhibit a step-wise change with depth into the tissue similar to the depth-dependent change in shear properties below the superficial zone reported previously (Buckley et al., 2010; Buckley et al., 2008). Since cartilage can generally be considered a composite of materials having differing properties (Asanbaeva et al., 2008; Mow and Guo, 2002), we tested the concept that immature articular cartilage, in particular, functions as a bilayer composite of superficial and deeper zone materials. We chose immature cartilage for this study due to the emerging clinical relevance of cartilage lesions in children and adolescents (Lauterburg and Segantini, 1994; Smith et al., 2005; Tallón-López et al., 2006; Stanitski et al., 1993; Sarpel et al., 2007; Stanitski, 1998; Stanitski and Paletta, 1998; Ure et al., 1992; Oeppen et al., 2004).

2. Methods

2.1. Superficial and deeper zone articular cartilage disks

Full-thickness cylindrical bovine articular cartilage explants (3 mm diameter, $n = 32$) including the intact superficial zone were harvested from the weight-bearing areas of the condyles and patellofemoral grooves of 1–2 week old calves ($n = 3$) within 24 h of death. Explants were equilibrated in 5% CO_2 in DMEM with 10% FBS, 10nM HEPES, 1nM sodium pyruvate, 0.1 mM non-essential amino acids, 0.4 mM proline, 20 μg/ml ascorbic acid plus antibiotics (Kurz et al., 2001). Explants were sliced perpendicular to the longitudinal axis into 200–400 μm-thick superficial zone disks including the articular surface (SZDs; $n = 32$) and deeper zone disks (DZDs; ~1300 μm; $n = 32$; see Fig. 1A schematic).

2.2. Cartilage thickness and biomechanical properties

Each disk ($n = 64$) was placed within the well of a loading chamber with an upper platen attached to an incubator-housed loading instrument (Frank et al., 2000). Thickness was measured individually for each disk by applying a slow compression ramp (20 μm/s ramp speed) with automated compression interrupt caused by an increase in offset load indicating contact between platen and disk. Utilizing the measured thickness of each SZD and corresponding DZD, we calculated the "depth position" of the center of each disk with respect to the articular surface (see Fig. 1A schematic).

To determine the mechanical properties of each SZD and DZD, three successive displacement-controlled ramp-and-hold compressions to final strains of 10% (200-s compression, 600-s hold), 12.5% and 15% (30-s compression, 300-s hold) were applied in unconfined compression. The resulting equilibrium loads (after stress relaxation) were used to compute the unconfined equilibrium moduli. At 15% final offset strain, each disk was then subjected to 3% dynamic strain amplitude at 1.0 and 0.1 Hz to compute the dynamic stiffnesses at each frequency (Kurz et al., 2001).

2.3. Biomechanical injury

After biomechanical characterization, each disk was equilibrated (20 min) and then subjected to injurious compression (unconfined) to a final strain of 50% at 100%/s strain rate ("injury"). During injury, the stress was continuously measured by the loading instrument (Frank et al., 2000). After 5 min of unconfined re-swelling, the disks were biomechanically re-characterized. Note in this context that some injured samples were macroscopically and microscopically damaged. As consequence, the geometrical shape of these samples was altered, and the re-characterization was to assess the effective macroscopic unconfined compression properties of the resulting injured explants.