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Cartilage and chondrocyte response to extreme muscular loading and impact loading: Can in vivo pre-load decrease impact-induced cell death?

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ABSTRACT

Background: Impact loading causes cartilage damage and cell death. Pre-loading prior to impact loading may protect cartilage and chondrocytes. However, there is no systematic evidence and understanding of the effects of pre-load strategies on cartilage damage and chondrocyte death. This study aimed at determining the effects of the pre-load history on impact-induced chondrocyte death in an intact joint.
Methods: Patellofemoral joints from 42 rabbits were loaded by controlled quadriceps muscle contractions and an external impacter. Two extreme muscular loading conditions were used: (i) a short-duration, high intensity, static muscle contraction, and (ii) a long-duration, low-intensity, cyclic muscle loading protocol. A 5-Joule centrally-oriented, gravity-accelerated impact load was applied to the joints. Chondrocyte viability was quantified following the muscular loading protocols, following application of the isolated impact loads, and following application of the impact loads that were preceded by the muscular pre-loads. Joint contact pressures were measured for all loading conditions by a pressure-sensitive film.
Findings: Comparing to cartilage injured by impact loading alone, cartilage pre-loaded by static, maximal intensity, short-term muscle loads had lower cell death, while cartilage pre-loaded by repetitive, low-intensity, long-term muscular loads has higher cell death. The locations of peak joint contact pressures were not strongly correlated with the locations of greatest cell death occurrence.
Interpretation: Static, high intensity, short muscular pre-load protected cells from impact injury, whereas repetitive, low intensity, prolonged muscular pre-loading to the point of muscular fatigue left the chondrocytes vulnerable to injury. However, cell death seems to be unrelated to the peak joint pressures.

1. Introduction

Injuries to cartilage are thought to trigger the development of a debilitating joint disease called post-traumatic osteoarthritis (PTOA) (Anderson et al., 2011; Dirschl et al., 2004). PTOA not only affects the quality of life of patients, it also imposes a substantial financial burden on the health care system, primarily because of the long-term conservative rehabilitation requirements and the large number of joint replacement surgeries performed today (Wieland et al., 2005).

Injuries to articular cartilage are often characterized by fissures in the extracellular matrix (ECM) (Chen et al., 2001a; Dirschl et al., 2004; Ewers et al., 2001; Krueger et al., 2003; Lewis et al., 2003; Rundell et al., 2005; Szczodry et al., 2009). Such fissures result in mechanical weakening and associated loss of protective properties for the chondrocytes. If ECM damage is substantial, it typically results in cell death and associated degeneration of the adjacent cartilage tissue (Shlopov et al., 1997). Most cases of osteoarthritis (OA) are associated with extensive cell death resulting in a decrease in the overall number of cells, and a concomitant failure of the remaining cells to maintain normal tissue homeostasis (Aigner et al., 2007; Blanco et al., 1998; Hashimoto et al., 1998). Thus, it is believed that preventing chondrocyte loss is a key factor in the maintenance of cartilage health and the prevention of the onset and rapid progression of OA.

Impact loading has been identified as a primary risk factor for cartilage damage and cell death (Duda et al., 2001; Isaac et al., 2008; Lewis et al., 2003; Milentijevic and Torzilli, 2005; Stolberg-Stolberg et al., 2013; Szczodry et al., 2009). Impact related joint injuries most often occur in car accidents and sports-related impact situations. In sport, accidents to joints typically occur after prior loading of cartilage in a game, while running or skiing. However, the effect of prior loading of cartilage on impact injury and associated cell death has not been studied systematically. Therefore, a realistic experimental set-up mimicking the effects of an impact injury in sport involves a pre-loading protocol prior to impact application.

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It is known that static and dynamic loading of cartilage alters the alignment of microstructural components in the tissue and also affects the load distribution between cartilage fluid and matrix phases (Arokoski et al., 1996; Morel et al., 2005; Mukherjee and Wayne, 1998; Park et al., 2003; Soltz and Ateshian, 1998, 2000). These changes may influence the mechanics of cells upon impact loading, and might produce vastly different amounts of cell deaths, depending on the detailed history of cartilage loading prior to impact. Static pre-loading prior to impact has been found to strengthen cartilage and reduce injury (Kim et al., 2012; Morel et al., 2005). On the other hand, the effects of cyclic loading on cartilage damage depend on the amplitude, frequency, and duration (Chen et al., 2003; Ko et al., 2013; Lucchinetti et al., 2002; McCormack and Mansour, 1997; Thibault et al., 2002; Wei et al., 2008; Zimmerman et al., 1988) of the load. While long-term cyclic pre-loading is thought to mechanically stiffen cartilage (Wei et al., 2008), the effects of short-term cyclic pre-loading prior to impact have yet to be explored.

Furthermore, pre-loading of cartilage followed by an injurious or impact load has been performed using externally applied loads through indenters or compression plates. Physiologically-relevant studies, where pre-loading is applied in an intact joint using the natural joint surfaces and muscular loading followed by impact loading, do not exist. But these are the conditions that occur in sport, thus it is important to understand the possible damage to the tissue and to identify pre-load strategies that can be incorporated into warm-up sessions to minimize cartilage damage and chondrocyte death upon possible impact.

Therefore, the aim of this study was to investigate the effects of joint pre-load history followed by impact loading on chondrocyte death. Experiments were performed in the rabbit patellofemoral joint using two extreme muscular loading conditions: (i) a short-duration maximal intensity muscle loading protocol similar to a 1 repetition, maximal isometric contraction, and (ii) a long-duration low-intensity muscular loading protocol similar to an exhausting running workout. Chondrocyte viability was evaluated following these muscular loading protocols and also following application of a controlled impact load immediately following these muscular pre-loading conditions. Patellofemoral joint contact pressures were also measured for all muscular and impact loading conditions in order to verify if contact pressures could explain potential cell death occurrence. We hypothesized (i) that the short-term and long-term extreme muscular loading conditions produce cell death, (ii) that static and cyclic muscular pre-loads of joints decrease the magnitude of cell death produced by an impact load, and (iii) that the location of cell death occurrence is related to areas of high joint contact pressures during impact loading. With this study, we should be able to answer two important questions: (i) can extreme (high force or long duration) muscular loading cause chondrocyte death in an otherwise healthy and intact joint, and (ii), can certain types of muscular conditioning protocols of the joint alleviate impact-induced chondrocyte death following impact loading.

2. Methods

2.1. Animal preparation and loading protocols

All testing was performed on patellofemoral joints from the hind limbs of skeletally mature (1–2 year-old) New Zealand white rabbits (Riemens, St. Agatha, Ontario, Canada) ($N_{rabbit} = 42$). Rabbits were anesthetized using a 5% isoflurane–oxygen mixture delivered through mask ventilation, and they were maintained at 2.5% isoflurane throughout the experiment. A bipolar nerve cuff electrode (a silicone tube of 3.4 mm in diameter and ~5 mm in length, with stainless steel wires on the inside for direct nerve contact) was implanted on the femoral nerve (Longino et al., 2005) of the experimental limb to allow for controlled stimulation of the quadriceps muscles.

After nerve cuff implantation, rabbits were fixed rigidly in a stereotaxic frame using bilateral bone pins in the pelvis and distal femur (Fig. 1a). The knee of the experimental limb was held at 95° of knee flexion. For impact loading, the distal femur was not fixed by bone pins. Instead, the tibia was held vertically by two clamps to allow for centrally-oriented impact loading on the patella using a drop-tower arrangement (Fig. 1b). Once fixed in this position, stimulation of the knee extensor muscles produced isometric knee extensor contractions with associated loading of the patellofemoral joint (Leumann et al., 2013; Ronsky et al., 1995). A strain-gauge-instrumented tibial restraining bar was positioned at the distal end of the tibia to measure the knee extensor forces (Herzog et al., 1998).

Controlled (frequency, duration and magnitude) electrical stimulation to the femoral nerve was delivered through a dual output Grass S8800 stimulator (Astro-MedInc., Longueil, Quebec, Canada). The α-motor neuron threshold of the knee extensor muscles was determined for each rabbit individually by gradually increasing the stimulation voltage of a 200 ms pulse train. Once two consecutive increases in stimulation magnitude did not result in a corresponding increase in force, it was established that all motor units of the knee extensor group were activated.

Once the stimulation threshold and the maximal stimulation magnitude were established, the patellofemoral joints were exposed to different types of muscular loading conditions, as explained in the following:

i. Maximal muscle contraction (10 s, continuous stimulation)
   The quadriceps muscles received a 10-second continuous supra-maximal electrical stimulation (4× α-motor neuron threshold, 150 Hz, 0.1 ms square wave pulse) of the femoral nerve (Herzog and Leonard, 1997) to produce the maximal possible isometric quadriceps loading of the patellofemoral joint.
ii. Sub-maximal muscle contraction (3000 s, cyclic stimulation)
   An exhaustive, sub-maximal muscle loading protocol was used to simulate the condition of continuous exercise. The quadriceps muscles were cyclically stimulated to produce 20% of the maximal isometric force for 1500 cycles (500 ms on, 1500 ms off, 0.1 ms square wave pulse). The stimulation current and frequency were adjusted throughout the experiment in order to maintain the muscle force at a constant level. In cases of extreme muscle fatigue (significant drop in muscle force for constant activation conditions), short rest periods (1–2 min) were allowed to regain the muscle force.
iii. Impact loading
   A 1.55 kg, 25 mm-diameter, flat-ended impactor was dropped from a height of 0.33 m onto the center of the patella through a custom-made drop tower in order to deliver a 5 J, centrally-oriented, blunt impact load to the patellofemoral joint.
iv. Impact loading 1-second after initiation of maximal muscle contraction (pre-1 s max, impact)
   Using a similar set-up as described for conditions (i, iii), a 5 J impact load was applied to the patellofemoral joint 1 s after the initiation of the supra-maximal isometric knee extensor contraction. The electrical stimulation was terminated immediately following the impact loading.
v. Impact loading within 5 min following submaximal muscle contraction (pre-3000 s submax, impact)
   Using a similar set-up as described for conditions (ii, iii), patellofemoral joints were first sub-maximally loaded by quadriceps muscle contractions for 50-minutes, followed by a 5 J impact load (5 min).

Rabbits were sacrificed immediately after the muscular and/or impact loading protocols by a barbiturate overdose using 2 ml Euthanyl (pentobarbital sodium, Biomeda-MTC pharmaceuticals, Cambridge, Ontario, Canada). All aspect of animal care and experimental procedures