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Physiologic deformational loading does not counteract the catabolic effects of interleukin-1 in long-term culture of chondrocyte-seeded agarose constructs

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ABSTRACT

An interplay of mechanical and chemical factors is integral to cartilage maintenance and/or degeneration. Interleukin-1 (IL-1) is a pro-inflammatory cytokine that is present at elevated concentrations in osteoarthritic joints and initiates the rapid degradation of cartilage when cultured *in vitro*. Several short-term studies have suggested that applied dynamic deformational loading may have a protective effect against the catabolic actions of IL-1. In the current study, we examine whether the long-term (42 days) application of dynamic deformational loading on chondrocyte-seeded agarose constructs can mitigate these catabolic effects. Three studies were carried out using two IL-1 isoforms (IL-1α and IL-1β) in chemically defined medium supplemented with a broad range of cytokine concentrations and durations. Physiologic loading was unable to counteract the long-term catabolic effects of IL-1 under any of the conditions tested, and in some cases led to further degeneration over unloaded controls.

1. Introduction

Articular cartilage is a specialized connective tissue that bears load and reduces friction across moving joints. It is composed of an extracellular matrix that contains no nerves or blood vessels and relatively few cells. Once injured, articular cartilage does not heal well, but often degenerates further, leading to pain and loss of function (Hangody and Modis, 2006). Tissue engineering offers great hope for expanding the range of treatment options by generating healthy replacement cartilage from a combination of isolated, living cells embedded in a scaffold carrier (Cima et al., 1991; Capito and Spector, 2003; Hung et al., 2004; Giannoudis and Pountos, 2005; Giannoni and Cancedda, 2006; Habibovic et al., 2006; Raghunath et al., 2007; Schulz and Bader, 2007).

In order to function within a defect site an engineered implant must have both the mechanical competency and the chemical fortitude to survive and flourish within an environment that is likely to contain potent catabolic mediators stemming from chronic inflammation (van den Berg and Bresnihan, 1999; Schiff, 2000; Lotz, 2001; Smeets et al., 2003). Interleukin-1 (IL-1) is a pro-inflammatory cytokine that has been shown to be elevated in osteoarthritis (Towle et al., 1997) and leads to cartilage degradation in *in vitro* tests (Ratcliffe et al., 1986; Morales and Hascall, 1989; Temple et al., 2006). The catabolic effects of IL-1 may be especially pronounced in underdeveloped engineered cartilage (Xu et al., 1996; Cook et al., 2000; Rotter et al., 2005; Lima et al., 2008), whose chondrocytes are not yet fully embedded in a dense chondroprotective cartilaginous extracellular matrix (Li et al., 2003).

As the interplay of mechanical and chemical factors is integral to cartilage maintenance and/or degeneration, it motivates researchers to examine the combined effects of chemical and mechanical stimuli (Mauck et al., 2003a, b). The chondrocyte-seeded agarose system has clear basic science and tissue-engineering applications in which both chemical and mechanical stimuli can be carefully controlled. Several short-term studies using an agarose culture model have suggested that applied loading may have a protective effect against the catabolic actions of IL-1 (Gassner et al., 1999; Honda et al., 2000; Xu et al., 2000; Agarwal et al., 2001; Chowdhury et al., 2001).

Chowdhury et al. (2001, 2003) have shown that dynamic loading counteracts IL-1-induced increase of nitric oxide (NO) and PGE_2 in chondrocyte-seeded agarose constructs. Mio and co-workers have reported that RNA expression of anabolic factors (aggrecan and type II collagen) in chondrocyte-seeded agarose constructs increases with application of dynamic loading for 24 h.

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Long-term culture of chondrocytes in agarose results in the formation of a functional matrix (Buschmann et al., 1992, 1995) and applied deformational loading can enhance development of tissue properties (Mauck et al., 2000, 2003a, b; Lima et al., 2007). The culture system preserves the chondrocyte phenotype by maintaining a physiologic three-dimensional environment and produces extracellular matrix components with a proteoglycan composition and corresponding Young's modulus similar to that of native cartilage (Mouw et al., 2005).

In the current *in vitro* study, we examine the effects of IL-1 on the mechanical and biochemical properties of engineered tissue and explore whether the long-term application of physiological levels of dynamic deformational loading on chondrocyte-seeded agarose constructs can mitigate these effects.

2. Materials and methods

2.1. Experimental design

Three studies were carried out in this set of experiments. In Study 1 ($n = 5$–7/group), we establish a broad range of response by culturing constructs for 28 days with or without dynamic deformational loading in a chemically defined (CD) medium with one of two IL-1 isoforms (IL-1α and IL-1β, 10 ng/mL). Based on these results, we selected the most potent isoform (IL-1α) for the remaining two studies. In Study 2 ($n = 6$–9/group), we examined the dependence on cytokine concentration by testing a logarithmic range from 0.01 to 10 ng/mL over 42 days in culture. In Study 3 ($n = 8$/group), we repeat the logarithmic range of concentrations; however, we restrict IL-1 exposure to periods of deformational loading only. Thus, in Study 3 IL-1 is present for 3 h/day (for both loaded and unloaded groups), while in Studies 1 and 2 IL-1 is present 24 h/day. The timelines of the studies are detailed in Fig. 1. Each study was performed independently, using individual cell isolations pooled from different animals, and repeated twice. Values reported were averaged across repeat studies.

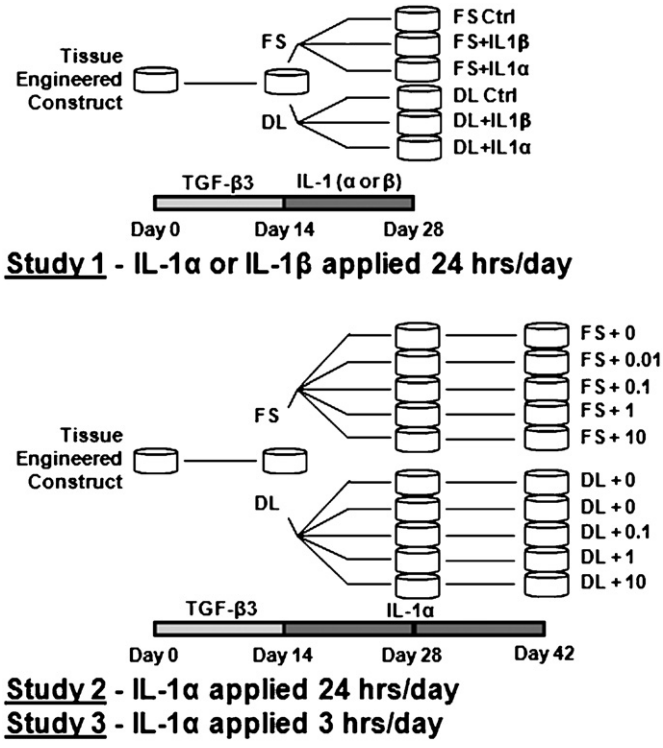

Fig. 1. Study 1: (testing IL-1 isoform dependence) tissue-engineered constructs were cultured for 14 days in a CD medium supplemented with TGF-β3. For the subsequent 14 days constructs were exposed to either IL-1α or IL-1β and either DL 3 h/day or maintained in FS without TGF-β3. Study 2: (testing IL-1 concentration): constructs were cultured for 14 days in CD medium with TGF-β3 and for the next 28 days were exposed to IL-1α in a logarithmic range of concentrations (0, 0.01, 0.1, 1, or 10 ng/mL) with or without loading. Study 3 (testing IL-1 exposure time): constructs were cultured as in Study 2, with the exception that IL-1α was added only during loading periods (3 h/day).

2.2. Cell isolation

Articular cartilage was harvested from bovine carpo-metacarpal (CMC) joints of freshly slaughtered 1–3-month-old calves. The cartilage tissue was digested in high-glucose Dulbecco's Modified Eagle's Medium (hgDMEM, 7.5 mL/g) with collagenase type IV (390 activity units/mL, Sigma, St. Louis, MO) for 11 h at 37 °C with stirring. The resulting cell suspension was filtered, combined, and cast into slabs with a final cell concentration of 30×10^6 in 2% agarose (Type VII, Sigma). The slabs were cored to final construct dimensions ($\varnothing 0.4 \times 0.23$ cm) and maintained in culture in one of the two medium formulations (described below) for up to 42 days depending on the study (Fig. 1).

2.3. Growth medium

CD medium consisted of hgDMEM supplemented with $1 \times$ PSF, 0.1 μM dexamethasone, 50 μg/mL ascorbate 2-phosphate, 40 μg/mL L-proline, 100 μg/mL sodium pyruvate, and 1X ITS+premix (insulin, human transferrin, and selenous acid, Becton Dickinson, Franklin Lakes, NJ). CD medium was further supplemented with 10 ng/mL of TGF-β3 (R&D Systems, Minneapolis, MN) for the first 14 days of culture. All culture media was changed every other day. This protocol has been shown to promote significant matrix elaboration that results in engineered tissue with native equilibrium modulus and proteoglycan content (Lima et al., 2007). The final mechanical and biochemical properties attained within the culture period can vary depending on the cell isolation, as is typical of the native tissue.

2.4. Interleukin supplementation

In Study 1, cell-seeded agarose constructs were cultured for 14 days in CD medium without IL-1. For the subsequent 14 days, constructs were exposed to either IL-1α or IL-1β at 10 ng/mL and either dynamically loaded (DL) 3 h/day or maintained in free swelling (FS). Mechanical testing and biochemical analysis was carried out as described below on days 0, 14, and 28.

In Studies 2 and 3, constructs were cultured for 14 days in CD medium without IL-1 as above. In Study 2, for the subsequent 28 days, the culture medium was supplemented with 0.01, 0.1, 1, or 10 ng/mL IL-1α and constructs were loaded or remained in FS. In Study 3, for the subsequent 28 days the constructs were exposed to 0.1, 1, or 10 ng/mL IL-1α during loading times only (for 3 h/day both FS and DL constructs were transferred to Petri dishes with new IL-1α-supplemented medium). For concentrations and time-courses see Fig. 1.

2.5. Loading protocol

Dynamic sinusoidal strain was applied at 1 Hz, with a nominal amplitude of 5% (10% peak-to-peak deformation) above a 10% tare strain, in unconfined compression with impermeable platens. Loading was applied continuously for 3 h/day, 5 days/week, beginning on day 14 (previously found to be optimal CD medium formulations (Lima et al., 2007)). Built-in compliance in the loading devices compensated for increasing stiffness in developing constructs, altering the load and displacement profiles and circumventing platen lift-off through the entire culture period as described previously (Lima et al., 2007).

2.6. Material testing

Cylindrical constructs were tested in unconfined compression using a custom computer-controlled testing system (Soltz and Ateshian, 1998). Samples were loaded to 10% strain at a strain rate of 0.05% strain/s, after an initial 0.02 N tare load. After achieving stress–relaxation equilibrium, the unconfined compression dynamic modulus G^* was measured by superimposing 2% peak-to-peak sinusoidal strain at 1 Hz.

2.7. Biochemical content

The biochemical content of each sample was assessed according to wet weight. Samples were digested in proteinase-K overnight at 56 °C and analyzed for either glycosaminoglycan (GAG) content using the 1,9-dimethylmethylene blue dye-binding assay (Farndale et al., 1982) or ortho-hydroxyproline (OHP) content via a colorimetric assay by reaction with chloramine-T and dimethylaminobenzaldehyde (Stegemann and Stalder, 1967), as described previously (Kelly et al., 2005). Cell viability (not shown) was measured via Live/Dead assay (Invitrogen, Carlsbad, CA).