**IL-1β enhances integrative articular cartilage repair**

+Khan, I M; Singhrao, S K; Gilbert, S; Gonzalez, L G; Mishra, A; Duance, V C; Archer, C W.

+Connective Tissue Biology Labs, School of Optometry, Cardiff School of Biosciences, Cardiff University, Museum Avenue, Cardiff, Wales, UK.

Senior author: archer@cardiff.ac.uk

Introduction: Lateral integration of articular cartilage following tissue reconstruction is problematic because there are many intrinsic barriers to fusion. Reduced chondrocyte cell density, increased cell death at the defect edges, chondrocyte aging or dedifferentiation and disruption of the collagenous network can all contribute to insufficient integration that can potentially lead to delamination of repair tissue. Principal amongst the impediments to cartilage integration is the presence of proteoglycan, which is rich in covalently bound glycosaminoglycans that are hydrophilic and whose electronegative properties resist applied compressive forces. The presence of proteoglycans inhibits chondrocyte migration, but enzymatic digestion using chondroitinase ABC, trypsin or hyaluronidase (especially in combination with collagenase) has been demonstrated to increase cartilage-cartilage integration, and in part this has been attributed to increased cell density at the interfacial region of the joined cartilages (1).

Digestion of the extracellular matrix (ecm) using purified enzymes not surprisingly induces changes in cell behavior, such as increased cellular proliferation and increased synthesis of ecm components (2,3). In addition, the affects on native tissue, in terms of short or long-term structural weakening are difficult to assess. Quite clearly modulation of the extracellular matrix is required to accelerate tissue integration, therefore we hypothesised that a short incubation of cartilage in the presence of pro-inflammatory cytokine interleukin-1β followed by a longer recovery phase (14 days) in the absence of the cytokine would enhance cartilage-cartilage integration through increased ecm degradation and temporary inhibition of proteoglycan synthesis. The effects of IL-1β on cartilage are known to be reversible and therefore we also predict that there would be no long-term damage to surrounding native cartilage.

Methods: Articular cartilage explants, from the metacarpal-pelageal joint of immature bovine steers were generated by first creating a 3mm diameter defect (disc) using a biopsy punch, then a 6mm diameter punch to generate a second, concentric defect (ring). The explants were equilibrated in standard medium DMEM high glucose supplemented with Gentamycin for 2 hours. Treated explants were equilibrated in standard medium DMEM supplemented with 50µg/ml ascorbate-2-phosphate and insulin-transferrin-selenium (ITS; Sigma) for a further 14 days. Histologic assessment of integration was made using toluidine blue staining of 5µm cut sections from 4% paraformaldehyde fixed explants that were processed for wax embedding. Adhesive properties of the disc/ring interface after two weeks in culture were assessed using a push-out test (n=4). Thickness of the sample was measured using callipers. A custom-made mechanical testing device in which a 'push-out rod' displaced the disc strength with a Lloyd LX material testing machine (Lloyd Instruments Ltd, Hants, UK). A computer activated micro-stepper controlled the displacement of the push-out rod (0.05mm/min), whilst a load cell (100N) coupled to the rod measured the push-out force. The adhesive strength was calculated from the maximum force measured at failure per unit of interfacial area from the ring.

**Results:** Ring/disc articular cartilage explants were generated using biopsy punches. We then performed an *in vitro* experiment to determine if an initial incubation of cartilage explants with IL-1β (10ng/ml) for 0-48 hours in DMEM alone, followed by culture for 14 days in ITS containing medium enhanced cartilage integration between the interface of ring and disc cartilages. We analysed the extent of integration using histological examination of toluidine blue stained sections, see Figure 1(left). Explants initially treated with a 24-hour incubation with IL-1β demonstrated the best visual evidence of integrative cartilage repair. Quantitative analysis of adhesive strength demonstrated a significant 6.3-fold increase in adhesive strength between 24 hour IL-1β treated explants versus untreated control explants (5.63±1.3N versus 0.89±0.27N, respectively; n=4; P<0.05), Figure 1(right).

**Discussion:** A short period of incubation of cartilage explants with IL-1β enhances integrative cartilage repair. Published studies show cytokine stimulation of articular cartilage induces the activation of catabolic enzymes but also results in the inhibition of proteoglycan gene synthesis (4). This is contrast to studies that use purified enzymatic digestion where proteoglycan synthesis is significantly increased as a response (2). Whether a temporary delay in proteoglycan synthesis, in combination with catabolic degradation, is a key fact in enhancing integrative repair is being currently investigated using quantitative RT-PCR.

**References:**

Figure 1. (Left) Toluidine Blue staining of ring/disc explants initially treated with IL-1β for the indicated times or left untreated, and then cultured for a further 14 days in medium supplemented with ITS. Integrative repair occurred throughout the tissue depth in IL-1β treated explants (12-48 hours). (Right) Graphical representation of the results of push-out tests to determine the adhesive strength of the ring/disc cartilages cultured in the absence or presence of IL-1β (10ng/ml; 24 hours; P<0.05).

Poster No. 1096 • 55th Annual Meeting of the Orthopaedic Research Society