locations) per condition (A-D) were cultured (DMEM) as follows: A, uninjured cartilage; B, mechanically injured cartilage (a single compression to 50% strain at 100%/sec strain rate, resulting in ~20 MPa peak stress); C, uninjured cartilage co-incubated with JC; D, mechanically injured cartilage co-incubated with JC. Samples were cultured for 6h, 1, 2, 4, 6, 8, 10, 12, 14 and 16 days.

Aggrecan from the cartilage disks was purified by guanidine extraction. For Western blot, medium and cartilage samples were pooled (i.e. 6 per timepoint), deglycosylated and analysed by quantitative Western blot using antibodies against aggrecanase generated ARGS and MMP generated FFGV neoepitope fragments. For glycosaminoglycans (GAG) analysis (DMMB, dimethylmethylene blue) each sample was measured separately, and Mann-Whitney rank sum test was used for statistics.

Results: The amount of GAG released into the medium increased over time in all conditions, with no significant differences in the cumulative amount of GAG released into the medium during the 16 day incubation between the conditions (A-D).

In medium from all the culture conditions (A-D), a high molecular weight (Mw = 294 kDa) ARGS fragment (most likely ARGS-GELE) was observed. Mechanically injured cartilage (condition B) did not release more ARGS fragments into the medium than uninjured cartilage (condition A), whereas cartilage co-incubated with JC (conditions C, D) released more ARGS fragments into the medium compared to uninjured and mechanically injured cartilage (conditions A, B) (Fig. 1).

MMP generated FFGV fragments were mainly found in medium where mechanically injured cartilage had been co-incubated with JC (condition D) (Fig. 1).

No ARGS or FFGV fragments were detected in the extract from the cartilage explants.

Conclusions: Mechanical injury alone does not increase the release of aggrecanase and MMP generated ARGS and FFGV fragments. The release of ARGS fragments into medium is increased when cartilage is co-incubated with JC, suggesting that co-incubation with JC increases aggrecanase activity in the cartilage. The release of FFGV fragments into medium is increased when mechanically injured cartilage is co-incubated with JC, suggesting increased MMP activity.

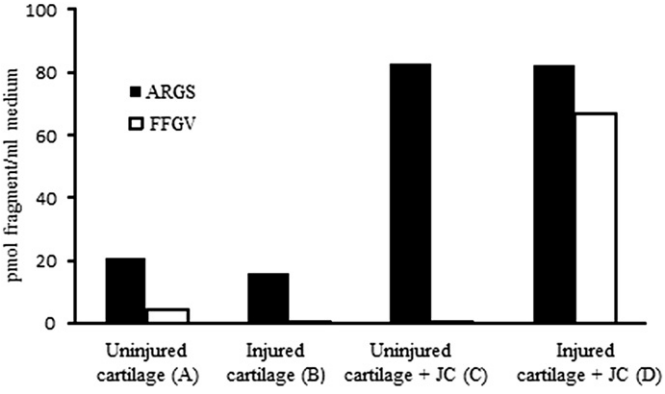

Figure 1. Total ARGS and FFGV fragments were quantified in medium from bovine cartilage by Western blot (n=1) using either ADAMTS4 or MMP-3 digested human cartilage-A1D1 fraction as ARGS-and FFGV standards. Cumulative values (16 days of incubation) are shown. JC, joint capsule.

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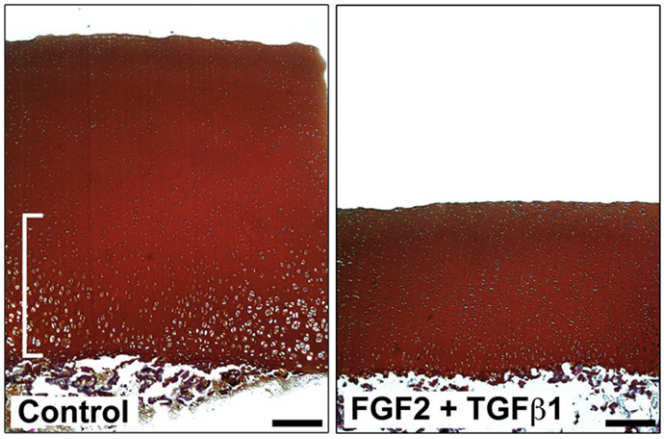
FGF2 AND TGFBETA1 INDUCE PRECOCIOUS POST-NATAL MATURATION OF ARTICULAR CARTILAGE: IMPLICATIONS FOR REPAIR OF OSTEOARTHRITIC LESIONS

I. Khan [1], C. Fellows [1], L. Francis [2], P. Theobald [1], C. Archer [1]. [1] *Cardiff Univ., Cardiff, United Kingdom;* [2] *Swansea Univ., Swansea, United Kingdom*

Purpose: Post-natal maturation of articular cartilage is a key developmental process that allows joint-specific adaptation of tissues to their local biochemical and biomechanical environment. Immature cartilage, a thick and relatively undifferentiated tissue undergoes a radical morphological transformation to generate a stiff and highly structured tissue that can potentially last a lifetime.

Cartilage in osteoarthritic lesions can be viewed as undergoing a reversal of maturation, in that molecular, cellular and extracellular features found in immature cartilage are re-expressed. Whilst it is true that the presence of articular cartilage-derived stem cells in osteoarthritic cartilage indicates that there is an inherent and viable cellular basis for tissue repair, the extent of repair is partially dependent upon recapitulation of post-natal developmental cues that induce maturation of cartilage.

We recently discovered that FGF2 and TGFβ1 induce precocious post-natal maturation of articular cartilage; rapidly restructuring immature cartilage in morphology, phenotype and function such that it is thinner, more differentiated and stiffer, see Figure.



The purpose of this study was to test the hypothesis that these changes represent accelerated postnatal maturation.

Methods: Histochemical and biochemical assays were used to confirm the nature of the morphologic changes that accompany growth factor stimulation of immature bovine articular cartilage explants cultured for 21 days in serum-free culture medium. Cellular proliferation was observed through bromodeoxyuridine incorporation assays. Collagenase activity was monitored by in situ zymography using a fluorescent substrate, DQ Gelatin. Growth factor-induced gene expression and changes in the collagen network were also quantitatively analysed.

Results: Growth factor-induced maturation in vitro occurs as a process of synchronised growth and resorption, with surface zone chondrocytes generating growth through increased cellular proliferation and highly regulated activity of matrix metallopeptidases stimulating resorption from the basal aspect of explants. Significant changes in collagen gene expression, and, structure through increased crosslinking correlated with an approximate 200% increase ($P<0.05$) in tissue stiffness. Using atomic force microscopy we observed that in vitro maturation caused a decrease in adhesion properties of the cartilage surface that matched the transition in adhesion values for freshly isolated immature and mature cartilage. The mean instantaneous frictional coefficient also increased during in vitro maturation matching an increases observed in freshly isolated immature and mature cartilage.

Conclusion: In vitro growth factor induced maturation of immature articular cartilage allows the accumulation of biomechanical and biotribological characteristics found in normal mature adult cartilage. Many of the problems associated with a lack of articular cartilage regeneration and repair are due to a deficit in our understanding of the basic developmental growth mechanisms of this tissue. To counter this, we describe a model system in which many of the important characteristics of the post-natal development transition are reproducibly exhibited. Our data opens out the possibilities to induce in vitro maturation of tissue engineered grafts prior to their implantation, or, to initiate maturational processes to heal osteoarthritic lesions.