FGF2 and TGFβ1 induce precocious maturation of articular cartilage
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INTRODUCTION
Articular cartilage maturation is the postnatal process whereby immature cartilage undergoes a morphological and biochemical transformation that adapt it to joint and site-specific biomechanical demands (1). The biochemical stimuli that initiate maturation have thus far remained elusive. However, we have recently discovered that in vitro culture with growth factors fibroblast growth factor-2 (FGF2) and transforming growth factor-β1 (TGFβ1) combinatorially induce precocious maturation of articular cartilage such that immature cartilage phenotypically and morphologically resembles mature cartilage (2), see Figure 1. Growth factor treated immature explants undergo a synchronized process of growth and resorption that results in an average 50 percent reduction in the height of explants, increased gene expression and activity of metalloproteinases MMP1, 13, 2 and 9 that promote resorption in the deep zone, increased expression of TIMP1-3 that regulate the extent of resorption and increased growth through cellular division at the surface of cartilage (2). In addition, collagen gene synthesis is significantly decreased, leading to an increase in number of pyridinoline collagen crosslinks, an unequivocal marker of maturing articular cartilage (3).

This study explores the hypothesis that growth factor induced maturation of immature cartilage generates biomechanical and biotribological properties similar to those found in mature cartilage.

MATATERIALS AND METHODS
Articular cartilage used in this study was obtained from the metacarpophalangeal (MCP) joints of immature (7-day-old) and mature (over 18-month-old) bovine steers. Growth factor induced maturation of immature bovine cartilage was performed by in vitro culture of 6mm diameter paired cartilage explants, isolated from the centre of the lateral aspect of the medial groove of metacarpophalangeal joints of immature bovine steers, for 21 days in the continual presence of 100ng ml⁻¹ FGF2 and 10ng ml⁻¹ TGFβ1 in serum-free medium supplemented with insulin-transferrin-selenium (ITS). Control explants were cultured in serum-free medium supplemented with ITS. Nanoscale biomechanical measurements were made using Nanowizard II (JPK Instruments) atomic force microscope (AFM). Nanoindentation force experiments were conducted capturing 100 indentation curves in a 10μm² scan area from the explant surface and all explants were analysed in triplicate. The frictional coefficients of cartilage samples were measured using a pin-on-plate tribometer using a specific lubricant for in vitro biotribological testing (British Standards: BS7271-7). Cartilage samples were preloaded at 0.1MPa for 120s prior to disc rotation to ensure consistent boundary lubrication. All data sets were check for normal distribution using the Shapiro-Wilk test and homogeneity of variances using Levene’s test. For parametric and non-parametric analysis we used one-way analysis of variance and Kruksal-Wallis tests, respectively.

RESULTS
The stiffness, as measured by AFM, of articular cartilage from the metacarpophalangeal joint increased 17-fold (P<0.002) as the tissue matured in vivo. A significant 5-fold (P<0.002) increase in elastic modulus was observed in growth factor treated immature explants when compared to untreated explants. Culture of immature explants in serum-free medium alone resulted in a 5-fold increase in stiffness when compared to uncultured immature explants, indicating that the process of maturation proceeds in vitro though at a significantly slower rate in the absence of exogenous growth factor addition. Measurements of adhesion, again using AFM, at the surface of explants revealed a decrease as cartilage matured, and this change appeared to correlate with the level of antibody labelling for PRG4 (lubricin). The process of maturation also led to a significant (P<0.05) increase in frictional coefficients in in vivo matured (4.6-fold) and in vitro matured (1.6-fold) cartilages compared to immature and untreated explants, respectively.

DISCUSSION
We have strong evidence to suggest that postnatal articular cartilage maturation is induced through the combinatorial activity of FGF and TGFβ growth factors. Our data demonstrates that the biomechanical and biotribological characteristics in in vitro growth factor treated immature cartilage are similar to those found in cartilages that have undergone maturation in vivo, and confirms our observations describing histological, biochemical and gene expression changes consistent with accelerated postnatal maturation in this in vitro system (2).

In adult diseased, injured or repaired joints, tissue morphology has more in common with immature rather than mature cartilage, and the associated decrease in biomechanical function compared to mature tissue potentially impedes repair and can lead to further degeneration. These data allow formulation of new hypotheses regarding the dissolution of the mature cartilage phenotype in diseased tissue and the potential reformation of this phenotype in diseased and repair cartilage using growth factors.

SIGNIFICANCE
Postnatal articular cartilage maturation is a critical developmental step in shaping the function of this tissue and there has been a gap in our knowledge of the biochemical stimulators of this process. Understanding how maturation is initiated and regulated will allow cartilage biologists and tissue engineers to control this process to enhance intrinsic repair in vivo and grow high quality cartilage in vitro.

REFERENCES

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Figure 1. FGF2 and TGFβ1 induce precocious maturation of articular cartilage. Immature cartilage explants from the MCP joint were cultured in the absence [A] or presence [B] of FGF2 and TGFβ1 (see Materials and Methods) for 21 days and then processed for histology and Safranin-O staining. We observed a 50% decrease in cartilage height that was caused by resorption of deep zone cartilage (white marker in [A]).

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