Tuning the differentiation of periosteum using biochemical and mechanical stimulation

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Introduction
High prevalence of osteoarthritis and poor intrinsic healing capacity of articular cartilage create a demand for cell-based cartilage repair strategies. It is possible to tissue engineer cartilage with almost native proteoglycan content, but collagen reaches only 15% to 35% of the native content. It is generally believed that the application of mechanical loading during culturing increases collagen content, and therewith mechanical properties. We hypothesize that sliding of an indenter over a construct would induce tensile strains (Fig 2A) that stimulate collagen synthesis, and consequently increase the mechanical properties of neo-formed cartilage.

Aim
In this study, we present the feasibility of chick periosteum as a model system to study periosteum-derived cartilage development. We hypothesize that differentiation of periosteum can be tuned towards tissue engineered cartilage with more desirable content, by selectively applying biochemical (e.g. growth factors) and mechanical stimuli.

Methods

Culture protocol
Periosteum was dissected from chick tibiotarsus (embryonic day 15) and embedded in between two agarose layers (Figure 1) [1].

The constructs were cultured for 1 week (n=3) while being subjected to three different experimental conditions:
1) Control
2) Addition of 10 ng/ml TGF-β1
3) Application of mechanical loading
4) Mechanical loading + 10 ng/ml TGF-β1

Mechanical loading
Sliding indentation was applied with a novel developed bioreactor system (Fig 2B) at a depth of 10% at 0.3 Hz for 4 hours/day and 5 days/week.

Results

Biochemical analyses
Biochemical analysis showed abundant expression of GAG in both the control and TGF-β1 group, which was not significantly different. Contrary, GAG content was lower at all time points in mechanically loaded constructs, but comparable to control and TGF-β1 group when TGF-β1 was added in addition to mechanical loading (Fig 3A).

Collagen content in the control and TGF-β1 group was similar compared to native periosteum. Contrary, collagen content was increased in the mechanically stimulated (with and without TGF-β1) groups at all time-points (Fig 3B).

Histology and immunohistochemistry
Histology confirmed the expression of GAG in the control and TGF-β1 group (Fig 4A,B). In mechanical loaded constructs, GAG was only expressed when TGF-β1 was added to the medium (Fig 4D). The collagen in control and TGF-β1 group was characterized as mainly type II (Fig 4E,F), whereas mechanical loading led to mainly collagen I expression (Fig 4G). When mechanical loading and TGF-β1 were combined, both collagen I and II were abundant in the tissue (Fig 4H).

Conclusion
The results support the hypothesis that differentiation can be tuned via biochemical and mechanical stimuli.
- Stimulation with TGF-β1 enhanced chondrogenic differentiation.
- Mechanical stimulation induced collagen synthesis, but suppressed chondrogenic differentiation.
- Simultaneous stimulation with TGF-β1 and mechanical loading increased both proteoglycans and collagen type II content. Hence, this study shows the feasibility to tune differentiation of chick periosteum, ranging from selective stimulation of sGAG synthesis to collagen synthesis by applying specific loading regimes. This offers great opportunities for engineering (fibro)cartilage with dedicated properties.

References