Depth-dependent Stimulation of Proteoglycan Synthesis by Sliding Indentation

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Introduction
Mechanical loading of tissue-engineered cartilage constructs enhances tissue properties, but the effect is still unsatisfactory, largely because the depth-dependent material and biochemical properties of native articular cartilage have not yet been reproduced. We propose that depth-dependent properties can be induced by applying dynamic depth-dependent, inhomogeneous strain fields during culture. Such strain fields can be obtained by compressing the tissue with an indenter, and subsequently moving this indenter over the construct. This study evaluates the hypothesis that this loading regime, which we named sliding indentation \cite{Van Donkelaar CC 2009}, induces a depth-dependent response of chondrocytes seeded in agarose.

Materials and Methods
Chondrocytes were isolated from full depth cartilage of calf carpometacarpal joints (n=4) and seeded at 5.10\textsuperscript{6} cells/ml density in 3% agarose rings. Subsequently, two groups were defined. The control group was cultured without loading and the other group was loaded by sliding indentation using a custom bioreactor for 2 hours per day, at an indentation depth of 10\%, such that the cells were indented once per second. After 7, 14 and 28 days of loading, rings were taken for analyses. They were sliced such that a 750 m thick superficial, 750 m thick middle and 1500 m thick deep layer resulted. Day 7 samples were used to evaluate gene expression levels for aggrecan by qPCR. Day 14 and 28 were used for quantitative evaluation of sulphated glycosaminoglycan (sGAG) content.

Results
Indentation resulted in a depth-dependent response in aggrecan gene expression levels (Fig. 1). Aggrecan gene expression was upregulated compared to unloaded controls in the superficial and middle zones. In the deep zone, no significant differences were visible.

![Fig. 1. Day 7 aggrecan gene expression levels in the different zones](image)

In the superficial and middle zone, sGAG levels of the loaded group were significantly higher compared to the control group. In the deep zone, no significant effect of loading was visible at both time points.

![Fig. 2. GAG levels per dry weight after 14 and 28 days.](image)

Discussion and Conclusions
This study provides the first evidence for the hypothesis that sliding indentation induces a depth-dependent response in chondrocytes seeded in agarose. This approach may therefore be promising to engineer cartilage constructs with inhomogeneous mechanical properties.

References

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Disclosures
The authors have nothing to disclose.