

Effect of mechanical load on matrix synthesis in scaffold free, de novo cartilage –like tissue

M. Stoddart, L. Ettinger, H. J. Häuselmann
Laboratory for Experimental Cartilage Research,
Center for Rheumatology and Bone Disease, Zürich

INTRODUCTION: The development of autologous *de novo* cartilage *in vitro* requires chondrocyte isolation and a period of pre-culture before reimplantation. Implantation of an immature three dimensional implant may lead to premature failure. With this in mind we investigated whether the application of cyclical load and shear would increase the matrix synthesis within scaffold free, de novo cartilage-like constructs.

METHODS: Chondrocytes were isolated from calf knee articular cartilage and cultured in DMEM:F12 with 10% Foetal calf serum. Chondrocytes were encapsulated in alginate [1]. Implants were produced by liberating the chondrocytes and seeding them into a rectangular mould containing space for 20 implants, each 5 mm in diameter and 1 mm deep. 0.5N of load was applied by way of a roller that is able to apply both load and shear. For mRNA analysis of Collagen II and Aggrecan, the mRNA was isolated by the procedure of Chomczynski and Sacchi [2] and quantification was performed by competitive PCR using a known quantity of plasmid DNA as standard. The resultant values were normalized to GAPDH expression. Glycosaminoglycan content was quantified by alcian blue precipitation and DNA content measured by Hoechst 33258 fluorescence.

RESULTS: RNA samples were taken hourly from implants subjected to load applied at a frequency of 0.276Hz for 4 hours. The expression of collagen II was increased within one hour of load (171% of control) as was aggrecan (202% of control). The increased expression of both genes peaked at 2 hours (collagen II 239% control, aggrecan 253% control) before returning to almost basal levels by four hours. The peak at two hours indicated a desensitization to the load being applied. Thus, further experiments consisting of 2 hours interspersed with a 2 hour pause. To investigate

longer term loading on matrix production, the implants were subjected to a loading regime consisting of 0.276Hz, 2 hours each morning and afternoon. After 4 days of load the samples were harvested and the RNA expression and GAG/DNA content was determined. Loaded samples demonstrated an increased expression of both matrix genes investigated (221 % collagen II and 292% aggrecan). There was also an increase of GAG/ DNA to 167% of the unloaded controls.

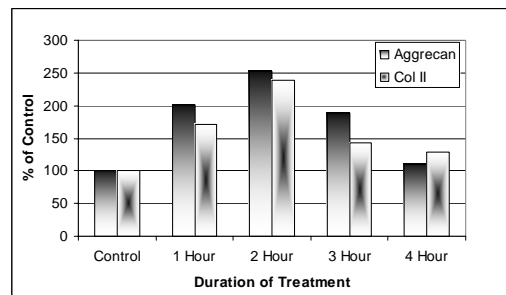


Fig. 1: Dynamic loading leads to an upregulation of both aggrecan and collagen II mRNA. This peaks at 2 hours before returning to normal levels at 4 hours.

DISCUSSION & CONCLUSIONS: The increase in incorporated matrix noted within 4 days of loading indicates the cyclic dynamic load, along with shear, may indeed be a viable method for increasing the matrix content of 3 dimensional implants prior to implantation.

REFERENCES:

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