

Mimicking the *in vivo* biomechanical environment

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INTRODUCTION:

Classical tissue engineering involves the use of competent cells, with a suitable scaffold and an enhancing stimulation. For tissue engineering of cartilage a period of three dimensional culture is often performed in order to pre-condition the tissue prior to implantation. The 3D culture is commonly improved by using a bioreactor system and many of these apply mechanical load, which has been shown to be beneficial for development of cartilage tissue. The ultimate determination of whether an implant is successful is derived from investigations involving animal studies. However these are time consuming and costly, as well as having ethics considerations. One step to reduce animal studies is the use of bioreactor systems in order to mimic the *in vivo* environment. This would enable a number of conditions to be investigated *in vitro*, prior to moving *in vivo* with the most promising conditions. We developed a ball-on-pin bioreactor which applies compression, rotation and shear (Fig. 1). This enables a biomechanical environment similar to that found within joints to be applied. The use of this system can give some insights as to what would occur in the *in vivo* situation.

We aimed to investigate the potential of SOX9 over-expression in the chondrogenic process of human adult bone marrow derived stem cells.

METHODS:

Unstimulated P3 human bone marrow MSCs were seeded into fibrin-polyurethane scaffolds (8 mm × 4 mm) at a density of 5×10^6 per scaffold. Cell-scaffold constructs were cultured in ITS+DMEM. As the *in vivo* implant site would not have TGF β and dexamethasone regularly applied, as would occur *in vitro* culture, we cultured the construct in the absence of any exogenous chondrogenic signals. The samples were subject to load following a protocol which would be comparable to a patient's rehabilitation protocol. One week no load, one week rotation only

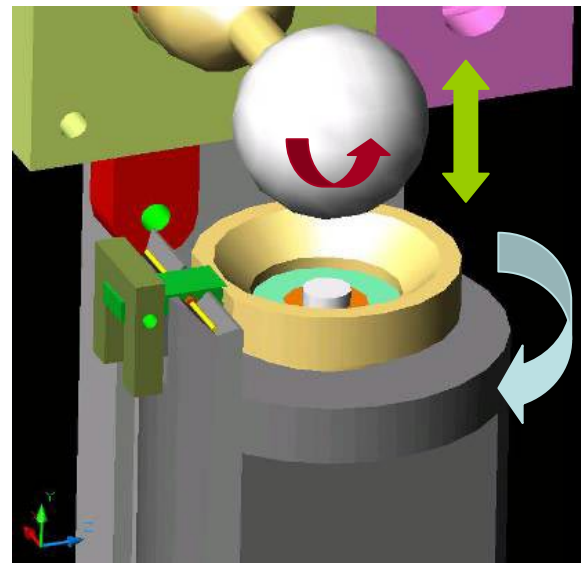


Fig1. Schematic of “Ball on Pin” bioreactor which allows for compression, and rotation of both the ball and the sample holder.

(cyclic passive motion) and one week rotation and compression (partial weight bearing).

RESULTS:

Other than proteoglycan 4 and COMP, load alone did not greatly increase chondrogenic gene expression. SOX9 over-expression increased the expression of cartilaginous associated genes ACAN, COL2, L-SOX5 and SOX6. The optimal expression profile was seen in cells exposed to both mechanical stimulation and SOX9 over-expression.

DISCUSSION & CONCLUSIONS:

This would suggest that *in vivo* the mechanical load applied during rehabilitation would improve chondrogenesis. This would not have been determined if the over-expression was investigated *in vitro* in the absence of mechanical load.

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