

Chondrogenesis of Mesenchymal Stem Cells is Differentially Regulated by Temporal Application of Dynamic Compression

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INTRODUCTION: Chondrogenesis of MSCs is thought to be regulated by the local mechanical environment. In the absence of chondrogenic growth factors, it has been demonstrated that dynamic compressive loading can enhance chondrogenesis of bone marrow derived MSCs¹. However we have recently demonstrated that dynamic compression can inhibit chondrogenesis of mesenchymal stem cells when applied at the onset of cytokine induced differentiation². The hypothesis under investigation in this study is that dynamic compression will enhance chondrogenesis of MSCs following transient exposure to TGF- β in free swelling culture.

METHODS: Porcine bone marrow derived MSCs (P3) were encapsulated in agarose (final concentration of 2%) at a cell density of 15×10^6 cells/mL. Agarose hydrogel constructs were maintained in a chemically defined chondrogenic medium supplemented with 10 ng/ml of TGF- β 3. For the first part of the study, dynamic compression (10% strain, 0.5Hz) was applied from day 0 (DC) or delayed until day 21 (DDC). Dynamic compression was applied for 5 days per week until day 42, with TGF- β 3 supplementation removed after day 21. In the second part of the study, TGF- β 3 supplementation was maintained for the entire 42 days of culture, and constructs were dynamically compressed as previously described. Controls were maintained in free swelling (FS) conditions. To assess construct functionality, samples were analysed biomechanically (equilibrium and dynamic modulus), biochemically (DNA, sGAG and collagen content in annulus and core of constructs) and histologically.

RESULTS: Application of dynamic compression to MSCs from day 0 (DC) was observed to inhibit chondrogenesis, as indicated by significantly lower levels of sGAG content at week 3 and 6 compared to FS controls. When TGF- β 3 supplementation was withdrawn after 3 weeks, sGAG content was greater in constructs

subjected to delayed dynamic compression (DDC) compared to free swelling controls, see Fig. 1. No difference was observed in sGAG levels between constructs subjected to DDC or FS conditions when TGF- β 3 supplementation was maintained for the entire 42 days of culture, see Fig. 2.

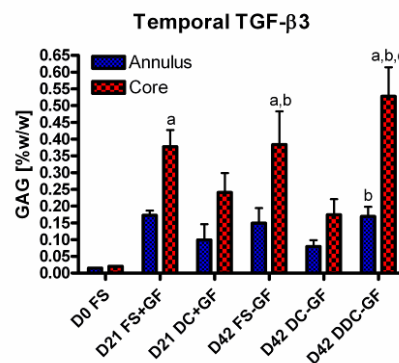


Fig. 1: sGAG content following removal of TGF- β after 3 weeks of FS culture.

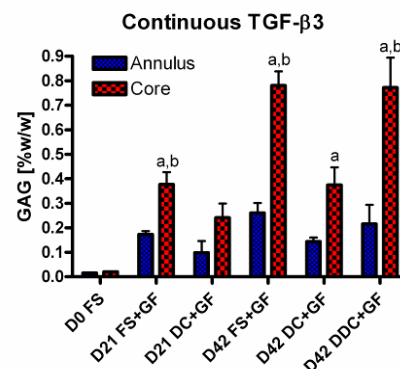


Fig. 2: sGAG content following continuous supplementation with TGF- β .

DISCUSSION & CONCLUSIONS: The results of this study suggest that MSC derived engineered cartilaginous tissues should be maintained for a sufficient period of time in FS conditions prior to implantation in a load bearing environment.

REFERENCES: ¹Huang, C.Y. et al. *Stem Cells*, 22, 313, 2004. ²Thorpe, S. et al. *Biochem Biophys Res Comm*, 377, 458, 2008.

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