Flow Perfusion Bioreactor Can Enhance Osteogenesis on a Collagen GAG Scaffold
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Introduction Flow perfusion bioreactors may be used to provide mechanostimulatory effects to cells and to improve cell distribution within a biomaterial. This study assessed the use of a flow perfusion bioreactor to improve cell distribution and osteogenesis within a collagen glycosaminoglycan (CG) scaffold.

Materials and Methods CG scaffolds were fabricated by a lyophilisation technique and cut to size (12mmØ) as previously reported1. 4x10⁶ hFOB 1.19 pre-osteoblast cells were seeded onto each scaffold and pre-cultured under standard conditions for 6 days. Bioreactor groups were exposed to 3 x 1 hr bouts of steady flow (1ml/min) with each bout being followed by 7 hrs of no flow (to prevent cellular desensitization) 2 for one day. Constructs were then cultured under osteogenic conditions for a further 28 days. Cellular distribution, mineralization, gene and protein expression of a number of bone formation markers and mechanical properties of the constructs were analyzed using techniques such as Haematoxylin and Eosin and alizarin red staining, real-time PCR and compression testing.

Results Both metabolic viability and cell number appeared similar between bioreactor and static culture groups. Histologically, cells in the constructs following bioreactor culture appeared in clusters which increased in distribution over time. In comparison, the static groups demonstrated a more uniform distribution, however, cells tended to aggregate on the periphery causing encapsulation. Osteogenesis was supported in both static & bioreactor groups. The early bone formation marker alkaline phosphatase gave a 3 fold increase in bioreactor groups at 21 days. The mid stage markers osteopontin and osteonectin showed similar trends with bioreactor groups providing higher expression levels earlier than the static groups. The late stage marker of bone formation, osteocalcin gave a 1.25 fold increase at 21 days. A 2 fold increase in alizarin red mineralisation was found in static groups at 28 days over bioreactor groups, which was due to the encapsulation effect. No difference was observed in mechanical strength between static or bioreactor groups.

Fig.1. Early and late stage osteogenic gene expression;

Discussion and Conclusions Flow perfusion bioreactors have been shown to stimulate osteoblasts by mechanoregulation1. We find that the bioreactor produced a more mature osteogenic state than static culture as well as discouraging peripheral encapsulation. This may be useful for in vitro applications as the presence of a capsule restricts nutrient diffusion and waste removal from a cell seeded construct. There was also no detrimental effect on the mechanical properties of the scaffold.

References

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