

Cell-free Collagen-based Scaffolds Enhance Healing Over MSC-seeded In vitro-engineered Bone Tissue Grafts

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INTRODUCTION: Recently, we have developed a collagen-calcium phosphate (CCP) scaffold [1] with improved mechanical properties compared to the collagen-glycosaminoglycan (CG) scaffolds [2] typically used in our laboratory for bone tissue engineering. The objective of this study was to compare the healing response of both scaffolds, when seeded with mesenchymal stem cells and maintained in osteogenic culture (tissue engineered constructs) and implanted directly (empty scaffold group) into a critical sized defect in a rat calvarium.

METHODS: CG and CCP scaffolds were fabricated using established lyophilisation procedures [1,2 respectively]. In the TE construct group, MSCs were harvested from the rat bone marrow using established techniques [3]. These cells were seeded on the CG and CCP scaffolds and maintained in osteoinductive factor-supplemented medium (100nM Dex, 50µg/ml ascorbic acid, 10mM β-glycerophosphate) for 28 days prior to implantation. A 7mm cranial defect was introduced into male white Wistar rats and scaffolds implanted with an empty defect group acting as control. Animals were sacrificed 4 and 8 weeks post surgery (n=9 per group). Defect areas and surrounding bone tissue was removed and analyzed using a quantitative X-Ray technique and micro-computed Tomography (µCT). After decalcification, histological sections were examined using Hematoxylin and Eosin staining and the degree of healing assessed.

RESULTS: Plain film X-Ray and µCT analysis of both cell-free scaffold groups showed increased healing and bone formation compared to empty defect and tissue engineered constructs. The CCP scaffolds showed greater healing than the CG scaffold groups with the non-seeded CCP scaffolds showed greatest healing with significantly higher levels (P<0.05) of mineralised tissue in the defects compared to all other groups including MSC-seeded CG and CCP scaffolds (TE constructs).

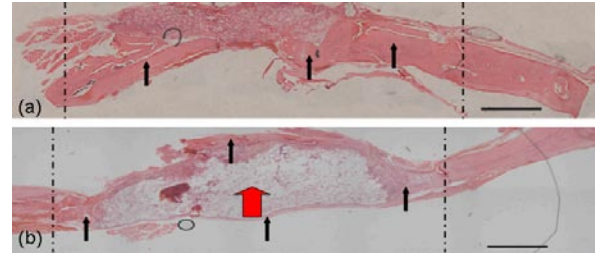


Fig. 1: In vivo healing of rat cranial defect in (a) empty CCP scaffold (b) tissue engineered CCP construct at 8 weeks post-implantation. Dashed lines indicate original (7mm) defect boundaries.

Histological analysis showed similar results but also revealed the potential cause of the lower levels of healing found in the tissue engineered constructs. Fig 1a shows almost complete bridging of the defect with high levels of new bone formation evident (black arrows) while Fig 1b shows the CCP scaffold cultured with MSCs. A ring of dense fibrous tissue and some bone is visible around the scaffold periphery (black arrows) with a major necrotic region present at the scaffold centre (red arrow).

DISCUSSION & CONCLUSIONS: This study has shown that two collagen-based scaffolds enhance bone healing in a rat critical sized defect model with the CCP scaffolds showing greatest healing after 8 weeks in vivo. Interestingly, the tissue engineering approach using MSCs cultured on the scaffolds showed the poorest healing. This is likely due to host fibrous tissue forming around the scaffold periphery. This results in an impermeable barrier to host cell infiltration and vascularisation following implantation in vivo. This suggests that issues with in vitro culture which can lead to avascular necrosis need to be overcome before clinical success will be achieved in the field of bone tissue engineering.

REFERENCES: ¹O'Brien FJ et al., *Biomater.* 2005. 26(4): 433-41. ²Al-Munajjed AA & O'Brien FJ. *J Mech Behav Biomed Mater.* 2009. (2) 138-146. ³Farrell E et al. 2006. *Tiss Eng* 12(3): 459-460.

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