Osteogenesis Increases with Larger Pores in Tissue Engineered Collagen-Glycosaminoglycan Scaffolds
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Introduction
When designing scaffolds for bone tissue engineering applications a major consideration is the mean pore size (1). A recent short term (7 day) study investigated the effect of pore size (85-325 µm) in collagen-glycosaminoglycan (CG) scaffolds and demonstrated that scaffolds with a larger mean pore size of 325µm facilitated increased cell attachment, proliferation and scaffold penetration (2). The objective of this study was to extend this original investigation into a long term study whereby the effect of mean pore size on osteogenesis and mineralisation was investigated to determine if larger pores increase osteogenesis in CG scaffolds developed for bone repair.

Materials and Methods
Scaffolds with different mean pore sizes were fabricated as previously described (3). These scaffolds were cultured with MC3T3-E1 pre-osteoblastic cells in osteogenic media (100nM dexamethasone, 50µg/ml ascorbic acid, 10mM B-GlyceroPhosphate) for 42 days and assessed every 7 days. Scaffolds were digested and cell density assessed by means of DNA quantification with Hoechst 33258. Scaffolds were assessed histologically using hematoxylin and eosin staining for cell infiltration, alizarin red staining for mineralisation and immunohistochemistry for osteopontin and osteocalcin expression. Two-way ANOVA followed by Holm-Sidak multiple comparisons were used to determine statistical difference in cell number between scaffolds.

Results
Cell viability was maintained over the 42 day culture period on all scaffolds. Scaffolds with a mean pore size of 325µm resulted in significantly higher cell number initially (p<0.031) (Fig. 1) in comparison to the other scaffold types. However, cell number significantly dropped at day 7 in scaffolds with larger pores and continued to decrease until day 21. Cell number significantly increased at day 7 in scaffolds with the smallest pore size of 85 m (p<0.050) and then drops off at day 14 indicating that differentiation begins earlier in scaffolds with larger mean pore sizes.

Histological analysis of the scaffolds demonstrated that mineralisation occurred in all scaffold types by day 42. However, mineralisation began at the earlier time point of day 14 in scaffolds with a mean pore size of 325 m in comparison to the other scaffold types where matrix was laid down at day 21. Cell infiltration was achieved with all scaffold types but cell aggregations remain around the edges of scaffolds with smaller mean pore size.

Discussion and Conclusions
Pore size has an effect on mineralisation within the scaffolds, with larger pores proving optimal as they facilitated successful migration, infiltration and early mineralisation beginning at day 14 in comparison to day 21 in other scaffold types. Mineralisation was seen to occur around the edges of the scaffolds with smaller pores and this may lead to encapsulation of the scaffold. These results combined with the previous study (2) prove that larger pores are optimal for bone tissue engineering.

References

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