Development of a Collagen-Nanohydroxyapatite Gene Activated Matrix for Bone Tissue Regeneration
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Introduction
Gene therapy for bone tissue regeneration requires a vector for the efficient delivery of plasmid DNA (pDNA) into cells [1]. In this study, nano-sized hydroxyapatite (nHA) particles were investigated as non-viral vectors for cellular transfection. Gene activated matrices (GAM) have been shown to provide enhanced, localised gene delivery and novel collagen-nHA composite scaffolds combined with the nHA delivery vector are investigated as a GAM suitable for bone tissue engineering.

Materials and Methods
A nHA suspension was optimized for use as a non-viral delivery vector, based on previous work [2]. The nHA samples were prepared in 50mM HEPES buffer with 140mM NaCl with or without 0.1 % Darvan dispersant agent (nHA +D, nHA –D) and filtered through a 0.2 μm filter before use. Two plasmids PmaxGFP and pGLuc (expressing the reporter genes green fluorescent protein (GFP) and luciferase) were pre-incubated in 10mM CaCl2 solution before addition of nHA. Coll-nHA scaffolds with 100 and 500 % nHA (S-100 and S-500) were soak-loaded with the nHA-pDNA complexes and seeded with rat MSCs [3]. Fluorescence microscopy showed expression of GFP from PmaxGFP transfected samples, while a luciferase assay (LumiFlexTM GLuc Assay Kit, New England Biolabs, USA) was applied to quantify expression of luciferase from PmaxGFP transfected samples, while a luciferase assay (LumiFlexTM GLuc Assay Kit, New England Biolabs, USA) was applied to quantify expression of luciferase from samples transfected with pGLuc. Group means were compared using ANOVA and the Tukey post-hoc test, significance was declared at p<0.05.

Results
The 2D study helped determine the preparation parameters of the nHA-pDNA solution which were necessary to ensure successful transfection. These included the presence of HEPES buffer, filtration of nHA particles and plasmid pre-incubation in CaCl2. Luciferase was expressed by cells seeded on all three scaffolds using nHA +D, nHA –D and a positive Lipofectamine control as the delivery vectors. Expression was sustained over the 10 day time period on the S-100 and S-500 coll-nHA composite scaffolds (significant vs. collagen control p<0.05). The expression of therapeutic genes from these composite scaffolds is envisaged to provide a highly adaptable template for bone tissue regeneration both in vitro and in vivo.

Discussion and Conclusions
This research has determined several factors which enable successful cellular transfection using nHA particles as non-viral delivery vectors. This has helped improve the efficiency of this method, which is a considerable drawback associated with this otherwise cost-effective, easy and safe technique. Gene activated 3D matrices allow for a more controllable and sustained delivery of pDNA and overcomes the limitations of traditional protein (growth factor) delivery systems by providing localised delivery and improved distribution of the over expressed protein by the transfected cells. The research has shown that collagen-nHA composite scaffolds are suitable for use as a gene activated matrix in bone tissue regeneration.

References

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