Novel Porous Fully Resorbable Calcium Phosphate Microspheres for Orthobiologic Applications

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Statement of Purpose: There is an on-going major shift in emphasis from tissue repair to tissue regeneration as a solution to the ever-growing need for long-term orthopaedic care. Minimally invasive procedures for bone repair and regeneration offer many benefits such as reduced trauma, especially for elderly patients. Microspheres have huge potential to encapsulate cells and many other types of biological components such as drugs including small molecules, nucleic acids, proteins etc and more importantly, can be administered easily through a syringe needle. Encapsulated components can then be manipulated and/or released at controlled rates for relatively long periods of time. As such phosphate-based glass biomaterials offer many advantages over other materials, as there degradation profiles can easily be controlled, from days, months to years (1).
This study investigated manufacture of bulk and porous microspheres from calcium phosphate-based glasses, their scale-up potential and stem cell encapsulation.

Methods: Several phosphate-based glass compositions were manufactured utilising the following precursors as starting materials: sodium dihydrogen phosphate (NaH_2PO_4), calcium hydrogen phosphate $CaHPO_4$), magnesium hydrogen phosphate trihydrate ($MgHPO_4 \cdot 3H_2O$) and phosphorous pentoxide (P_2O_5), acquired from Sigma Aldrich, UK. The precursors were mixed together and transferred to a 100-ml volume Pt/5% Au crucible (Birmingham Metal Company, UK), which was then placed in a furnace (which had been preheated to 350 ºC) for half an hour for the removal of H_2O. The salt mixtures were then melted in a furnace at 1150 ºC for 1.5 h. The resulting molten glass was poured onto a steel plate and left to cool to room temperature. The glass was then ground and sieved into varying particle size ranges for manufacture of bulk and porous glass microspheres. SEM analysis was conducted to confirm microsphere morphologies and cytocompatibility studies were conducted utilising human mesenchymal stem cells.

Results: Phosphate-based glass microspheres of varying size ranges were successfully manufactured. Early trials confirmed that bulk microsphere production was feasible showing 95% efficacy in yielding spherical morphologies as shown in Figures 1A. Follow-on manufacturing trials showed that porous microspheres could also be manufactured, and at similar yields to the bulk microspheres, both from a single stage manufacturing process (see Figures 1B and 1C).
Further feasibility studies also showed human mesenchymal stem cells (hMSC) not only attached to the microspheres, but also migrated to reside within the pores of the porous microspheres (Figure1D).

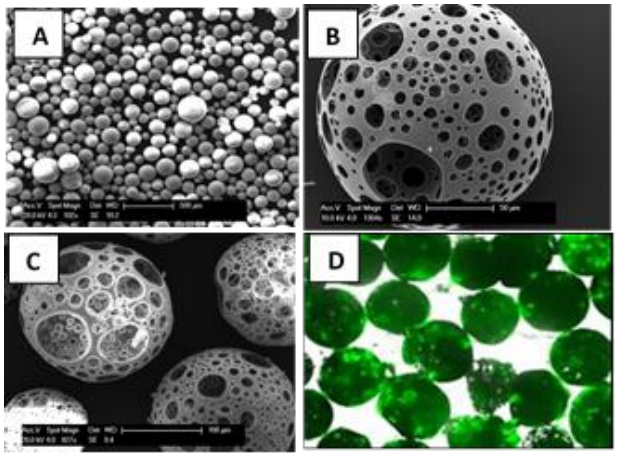

Figure 1: A) Shows bulk microspheres; B, C) show the levels of porosity achieved; and D) hMSCs attached.

Conclusions:
Bulk glass microspheres with diameter ranges from 60-350µm have been produced. Initial trials also showed successful manufacture of highly porous microspheres with porosity levels of over 80% and scale-up levels of between 1 – 3 kg per hour can be achieved. Moreover, these early indications suggest that porosity levels could be controlled to a certain degree.
Cell culture studies showed successful attachment and incorporation of human mesenchymal stem cells within their porous structure. The added advantage of their porous network would allow diffusion of nutrients, oxygen and waste products, along with the capacity of vascular penetration.
These early trials have shown the potential of loading these microspheres with other biological components and factors to provide a biotherapeutic effect (2) for the repair and regeneration of bone tissue, and could deliver the next generation of orthobiologic materials.

References:
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