Hydroxyapatite-calcium sulfate biphasic microspheres as a growth factor carrier for sustainable release

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Statement of Purpose Bioactive ceramic microspheres have been developed for regeneration of defective bones and have been used as a growth factor carrier by loading bioactive molecules (e.g., bone morphogenetic protein-2 (BMP-2)) in order to accelerate bone healing processes [1]. However, the release behavior of externally or internally loaded growth factors in bioceramic microspheres has often shown initial bursting and short-term release period [2]. Therefore, in this study, we have developed hydroxyapatite (HA) - calcium sulfate biphasic microspheres that allow sustainable release of loaded growth factors for the prolonged period while maintaining structural integrity as a space filler in the defect.

Methods: Calcium sulfate dihydrate (CSD) was mixed with bone cement powders (a mixture of of α-tricalcium phosphate (α-TCP) and tetracalcium phosphate (TTCP)), hardening liquid (1.33M sodium phosphate solution with 13.3 wt% citric acid) and 25vol% BMP-2 solution (0.3mg/ml). Subsequently, the composite microspheres were obtained by emulsifying the paste in oil and then were aged in oil at 37℃ for 3 days to fully convert bone cement to HA. The morphology and phase of all samples were investigated by SEM and XRD. Microspheres were immersed in PBS at 37℃ with shaking at 60 rpm. The amount of released BMP-2 under physiological conditions was determined by UV spectrophotometer.

Results: HA microspheres converted from bone cement exhibit the smooth external surface with internal isolated pores (**Fig. 1A**). With 50wt% CSD, microspheres appear to have rod-like CSD crystals both external and internal surfaces (**Fig. 1B**). After 4 weeks of immersion in simulated body fluid (SBF) solution, the morphology of pure HA microspheres (**Fig. 2A**) displayed minimal difference from its intact morphology in **Fig. 1A**, whereas 50wt% HA-CSD microspheres became porous through dissolved CSD crystals that created the long and narrow open pore channels in the microspheres (**Fig. 2B**). HA microspheres initially consisted of mainly HA and a small amount of α-TCP remnant and after 4 week immersion in SBF, was fully converted to HA (**Fig. 2C**). On the other hand, all CSD crystals in 50 wt% HA-CSD were dissolved so that the resultant microspheres became pure HA (**Fig. 2D**). The release of BMP-2 loaded into HA microspheres was saturated after ~2 weeks, whereas BMP-2 from HA-CSD microspheres was released up to 60 days, exhibiting gradual release over initial 30 days without significant bursting effects (**Fig. 3**).

Conclusions: HA-CSD biphasic microspheres were successfully fabricated through the oil emulsion method in conjunction with SBF immersion. The CSD phase was found to create the open pore channels in the microspheres through degradation in SBF for ~ 4weeks. This sacrifice of the CSD phase in biphasic microspheres allows prolonged and continuous growth factor release from the microspheres, implying great potential as a growth factor carrier for bone defects.

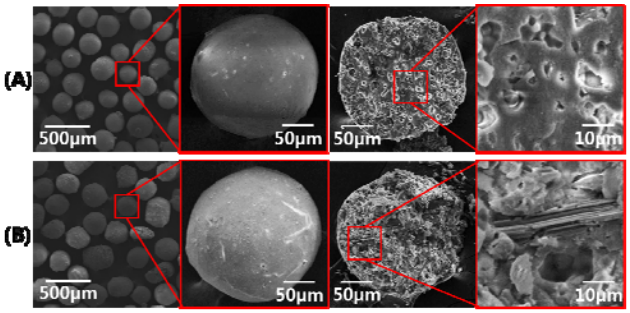

Figure 1. SEM images of surface and cross section of (A) 0wt% and (B) 50wt% CSD microspheres.

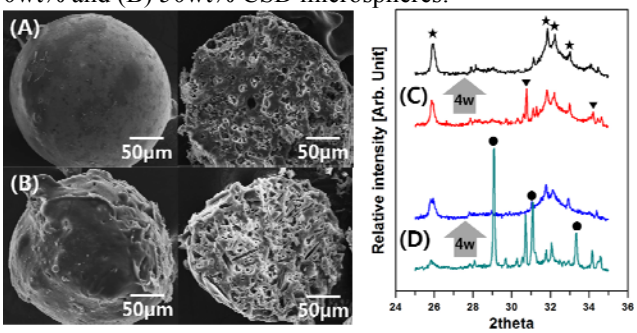

Figure 2. Surface and cross section morphologies of (A) HA and (B) 50wt% HA-CSD microsphere after immersion in SBF for 4 weeks, and XRD patterns of (C) HA and (D) 50wt% HA-CSD microspheres before (lower) and after (upper) the SBF test. (●: CSD, ▼: α-TCP, ★: HA)

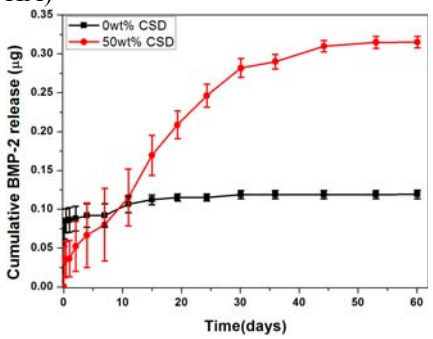

Figure 3. Cumulative BMP-2 release from HA and 50 wt% HA-CSD microspheres. (n=3)

References:

[1] Park. J. H. Tissue Eng Pt B-Rev. 2013;19(2):172-190
[2] Ginebra. M. P. Adv Drug Deliv Rev, 2012;64:1090-1110