Accelerated bone regeneration of BMP-2 loaded hydroxyapatite microspheres for bone filler applications

Jae-Uk Baek, Hyun-Do Jung, Tae-Sik Jang, Sung Won Kim, Min-Ho Kang, Juha Song, Hyoun-Ee Kim.
Department of Materials Science and Engineering, Seoul National University, Seoul, Republic of Korea

Statement of Purpose: Hydroxyapatite (HA) microspheres have gained attention as a bone filler in defective bones because of good bioactivity, chemical and mechanical stability and ease of use [1]. In particular, our previous study [2], we have successfully developed the low-temperature fabrication method of HA microspheres by converting bone cement microspheres into hydroxyapatite in oil which have great potential as a carrier of bioactive molecules. In this study, bone morphogenetic protein-2 (BMP-2) has been directly incorporated into HA microspheres during the fabrication, which allows BMP-2 to be loaded into the internal part of HA microspheres. In order to confirm the effect of released BMP-2, BMP-2 loaded HA microspheres have been evaluated via *in vivo* tests using a rabbit calvaria defect model as compared to pure HA microspheres.
Methods: Bone cement powders were prepared as the 2:3 mixture of α-tricalcium phosphate (α-TCP) and tetracalcium phosphate (TTCP) with the hardening liquid, 1.33M sodium phosphate solution mixed with 13.3 wt% citric acid. BMP-2 loaded microspheres were obtained by emulsifying bone cement pastes (a mixture of 1 g cement powder, 0.45 ml hardening liquid and 45 μg BMP-2) in oil for 10 min. The solidified microspheres were kept in oil at 37℃ for 3 days. The morphology and composition of microspheres were assessed by SEM and XRD. A rabbit calvaria defect model was used for *in vivo* animal tests where polycarbonate (PC) tubes (ϕ 6 mm x 4 mm) filled with microspheres were implanted on calvarias of New Zealand white rabbits (**Fig. 3A**) for 4 weeks and 8 weeks and were observed by Micro CT.
Results: Spherical microspheres having a diameter of ~ 250 μm were uniformly fabricated with nano-sized internal pores (**Fig. 1A**). Through aging in oil for 3 days, α-TCP and TTCP bone cement was converted to HA (**Fig. 1B**). Before loading BMP-2, to visualize how the loaded drug was incorporated into the individual microspheres, the cross-section image of a HA microsphere with green fluorescent protein (GFP) was observed, where GFP was well loaded in the internal part of the sphere (**Fig. 2A**). The release behavior of BMP-2 from the microspheres exhibits initial burst and the gradual release that lasted for 45 days (**Fig. 2B**). The bone regeneration accelerated by BMP-2, was clearly observed from the *in-vivo* test as shown in **Fig. 3B**. After 4 week implantation, the total amount of new bone formed within BMP-2 loaded HA microspheres appeared larger than that within bare HA microspheres. Moreover, bone ingrowth from the interface between the implant and bone was further progressed within BMP-2 loaded HA, where the depth of regenerated bone from the interface within BMP-2 loaded HA microspheres was found to be twice larger than that within bare HA.

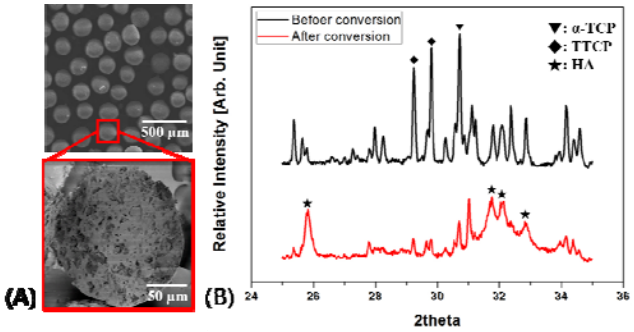

Figure 1. (A) Morphology of HA microspheres with porous cross-section surface and (B) XRD patterns of microspheres before and after conversion.

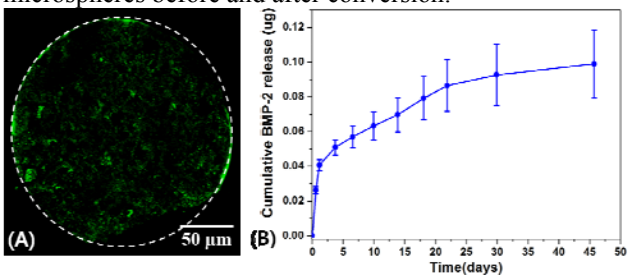

Figure 2. (A) Visualization of loaded drug (GFP) into the internal region of HA microspheres and (B) release behavior of BMP-2 from BMP-2 loaded HA microspheres up to 45 days (n=3).

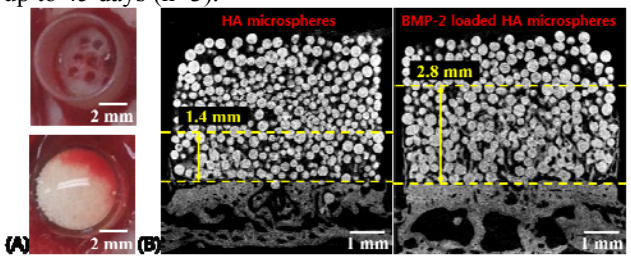

Figure 3. (A) Optical images of implanted HA microspheres on the rabbit calvaria using PC tubes and (B) micro CT images of implanted HA bare (left) and BMP-2 loaded (right) microsphere after 4 weeks of healing.

Conclusions: BMP-2 loaded HA microspheres were successfully fabricated through the oil emulation process. BMP-2 was loaded in the internal region of the microspheres, which allows gradual release of BMP-2 for a prolonged period. The *in vivo* test proved accelerated bone regeneration of BMP-2 released from HA microspheres, implying great potential of HA loaded microspheres as bone filler applications.

References:

[1] Ginebra. M. P. Acta Biomater. 2010;6:2863-2873
[2] J.-U. Baek. et al, Abstract #113 ©2014 Society for Biomaterials