Sustained release of biological molecules from calcium phosphate microspheres

Jae-Uk Baek, Hyun-do Jung, Tae-sik Jang, Sung-won Kim, Min-ho Kang, Hyoun-Ee Kim.

Department of Materials Science and Engineering, Seoul National University, Seoul, Republic of Korea

Statement of Purpose: Bioactive ceramic microspheres have been used in defective bones since they have high surface area, advantages in bone ingrowth, and excellent injectability. Drugs or biological molecules are often utilized in order to accelerate bone healing rate. However, ceramic processing which mostly requires heat treatment has bioactive molecules limited to be applicable on the surface of ceramic. Thus, in this study, we have achieved promising hydroxyapatite microspheres with biological molecules inside and significantly extended release period.
Methods: Calcium phosphate cement powders were prepared as the 2:3 mixture of α-tricalcium phosphate (α-TCP) and tetracalcium phosphate (TTCP). As the hardening liquid of bone cement, 1.33M sodium phosphate solution with 13.3 wt% citric acid was used. Green fluorescent protein (GFP) loaded microspheres was obtained by mixing 1 g cement powder and 0.3 ml hardening liquid and 0.1 ml GFP of 0.3mg/ml concentration for 30sec and then was immediately emulsified in olive oil for 10 min. The solidified microspheres were kept in oil at 37℃ for 3 days, which allows conversion to HA. For a control group, GFP was loaded only on the surface of microspheres by immersion in GFP solution. The morphology of all samples was assessed by SEM. The amount of GFP released was determined by UV spectrophotometer. GFP image was observed by confocal laser scanning microscopy (CLSM).
Results: In the cross section of the microsphere, porous structure was observed (Fig. 1 A) and HA needle was well grown (Fig. 1 B). Without direct contact to water, HA was well formed with moisture in the microspheres. This isolation in oil during the transformation is important because large amount of loaded molecules can be lost if the conversion takes place in the SBF solution. With minimum loss, GFP was loaded inside of microspheres and on the surface as well (Fig. 2A-C). After 4weeks of release in PBS, there was still green fluorescence detected inside of the microsphere (Fig. 2B), but there was few amount of GFP on the surface of the microsphere (Fig 2D). This implies that GFP loaded inside of the microsphere could be released more than one month. After that, GFP release kinetic of two kinds of sample was measured and release pattern was turned out to be totally different. More amount of GFP was steadily released from the inside during one month, but GFP on the surface was mostly released within a few days.(Fig. 3) This is because the inside of microspheres has very high surface area of nano HA needle which is known to strongly bind proteins and continuously release it. [2] Therefore, HA needle formed inside of the microspheres played a key role in prolonged release period.

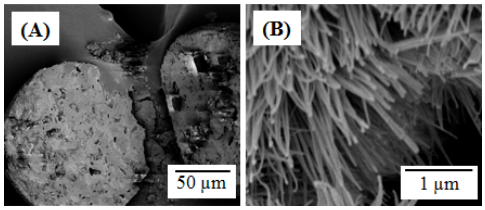

Figure 1. (A) Low and (B) high magnification of microstructure of the microsphere.

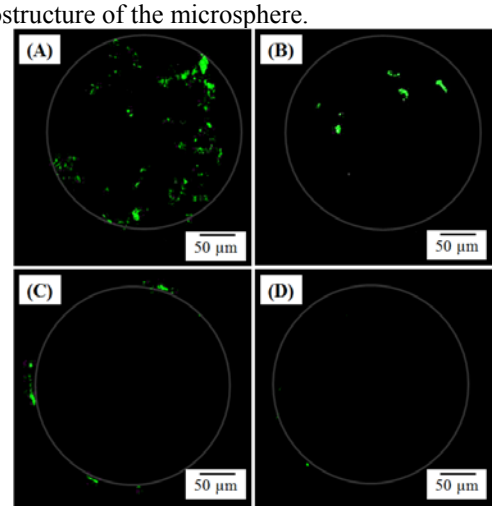

Figure 2. Cross section images of microspheres containing GFP (A) inside and (C) on the surface. (B)(D)Their cross section images after 4weeks of release. (Circles are approximated microspheres boundary)

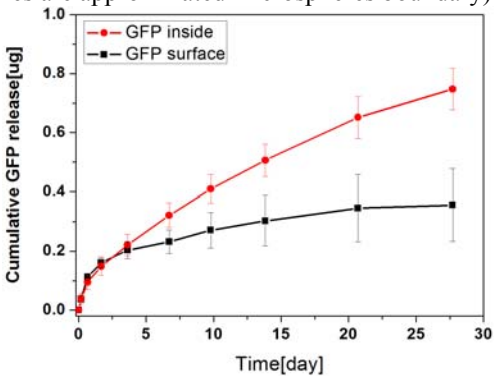

Figure 3. Release profile of GFP loaded inside of and on the surface of microspheres. (n=3)
Conclusions: By incorporating GFP in the bone cement paste, HA microspheres steadily releasing biological molecules for a long period of time were successfully obtained. Nano HA needle strongly bound and slowly released proteins. Therefore, we were able to satisfy biomedical needs requiring continuous effect of drugs or bioactive molecules.
References:
[1] Ginebra. M. P. Acta Biomater. 2010;6:2863-2873
[2] Ginebra. M. P. Adv Drug Deliv Rev, 2012;64:1090-1110