Sustained Bone Morphogenetic Protein 2 Delivery from Densified Titanium for the Hard Tissue Engineeering

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Statement of Purpose:
Titanium (Ti) and its alloys are used extensively for orthopedic and dental implants, owing to their good mechanical properties, chemical stability, and biocompatibility [1]. Recently, biomedical implants were improved osteoinductivity incorporating with growth factors, such as bone morphogenetic proteins (BMPs). Since these growth factors can promote cell proliferation and differentiation, they are effective in supporting tissue regeneration and healing [2]. However, growth factors deposited on the implant surface are often easily released with a rapid burst [3]. Therefore the osteogenic effect is minimal. In this study, we fabricated BMP-2-embedded Ti implants using new techniques and improve the potential for embedment of growth factors.

Methods: For growth factor loading, porous Ti discs were soaked in BMP-2 solution (50μg/ml) in a vacuum, and then air-dried. After drying, coated porous Ti discs were pressed by uniaxial press. And the cell attachment was evaluated. The BMP-2 release test was performed in 5 ml PBS at 37 °C for 140 days. The *in vitro* biological properties were determined by observing the MC3T3-E1 pre-osteoblast cell attachment, proliferation and differentiation. Ti embedded with BMP-2 transplanted into critical size calvaria defect (12 mm) of white rabbit and evaluated by micro-CT over 6 weeks.

Results: Fig. 2 shows the in vitro release kinetics of bare, porous and embedded Ti for 140 days. From the release kinetics it is clearly evident that the BMP-2-embedded Ti remarkably reduces the initial burst release of Ti compared to porous Ti. This release trend can be explained on the basis of the narrow pores like an ant tunnel when pressed. These narrow pores allows for a more controlled and long-termed release. The cells on as-received Ti showed round morphologies with minimal spreading (Fig. 2(A)). By contrast, the cells adhered and spread well on BMP-2-embedded Ti surfaces. Slightly higher cell density was observed on BMP-2-embedded Ti compared to bare Ti at 5 days. However, significantly higher ALP activity was observed on BMP-2-embedded Ti compared to that of as-received Ti suggesting that BMP-2 enhances the bioactivity of the samples (Fig. 2(B)). After 6weeks, the same general trend was observed as shown in figure. . For BMP-2-embedded Ti, a more complete bone healing response was found, as the defect void continued to be filled by new bone formation after initially bridging the segmental gap after 6 weeks.

Conclusions: The present results show promising potential for the application of dense titanium embedded with BMP-2 as a novel metal biocompatible device for sustained delivery of proteins such as growth factors and drugs. And promising levels of bone regeneration have been observed, evidenced by the critical size calvaria defect animal study. Thus, new insights into enhanced bone regenerative biomaterials are being developed by following the principles of nature tissue formation.

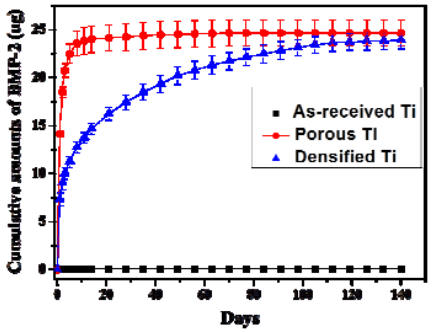

Fig 1. Release patterns of BMP-2 from Ti implants

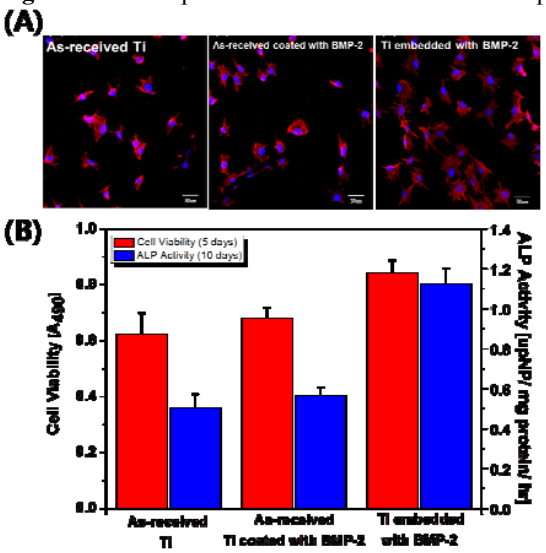

Fig 2. (A) Cell attachment (after 3 h of culturing), and (B) viability and differentiation of the MC3T3-E1 cells cultured on Ti implants for 5 and 10 days

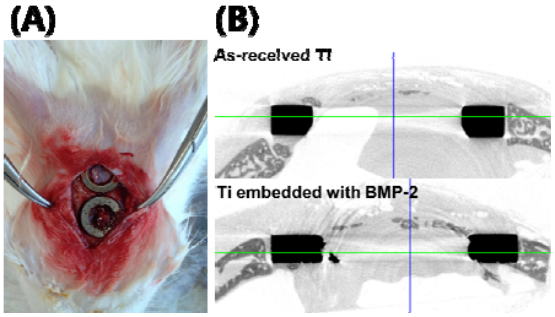

Fig 3. (A) Optical image of implantation and (B) micro-CT image of calvaria defect model after 6 weeks

References:

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2. Xiao YT et al. Biochemical and Biophysical Research Communications 2007; **362**; 550-553
3. Kitajima T et al. Biomaterials 2007; **2**; 1989-1997