Osteogenic Potential of BMP2-loaded Nanocomplex/ Hydroxyapatite Layer on Titanium Surface

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Introduction: Hydroxyapatite (HA) coating technique has been well-known as the best choice for most dental and orthopaedic applications due to their osteoconductive properties. Recently, biomedical implants can be made osteoinductive by incorporating an osteogenic agent, such as bone morphogenetic proteins (BMPs). Since these growth factors can promote cell proliferation and differentiation, they should be effective in supporting tissue regeneration and healing. However, BMP-2 deposited on the surface of calcium/phosphate layer is often easily released with a rapid burst. Therefore the osteogenic effect is minimal. Various attempts have been carried out for their potential to serve as a suitable delivery vehicle of growth factors to target site. In this study, we employed a BMP2-loaded nanocomplex (NC) deposited on the calcium/phosphate layer as a carrier of BMP-2. The osteogenic efficacy of this system was evaluated in vitro.

Materials and Methods: Commercially pure titanium (Ti) discs (10 mmΦ x 0.5 mm) were cleaned in an ultrasonic washer alternately using acetone, alcohol and distilled water. For the surface treatment, Ti plates were soaked in oxidization solution of hydrogen peroxide, ammonium hydroxide, and distilled water at 80^{o}C for 5 min, then sonicated in 90% butanol for 30 min, and finally vacuum-dried. The Ti samples were then immersed in a simulated body fluid (SBF; NaCl: 8.035 g; $CaCl_2$: 0.292 g; $Na_2HPO_4{\cdot}2H_2O$: 0.231 g; KCl: 0.225 g; $NaHCO_3$: 0.355 g; Na_2SO_4: 0.072 g) buffered with Tris (pH 7.4), for 7 days at 37^{o}C. BMP-2 nanocomplex was fabricated from the charge-charge interactions between chondroitin sulfate and BMP-2. The rhBMP2-loaded NC was incorporated onto the apatite-coated surface in the SBF solution. The surface microstructure and compositional changes evaluated by scanning electron microscopy (SEM), energy dispersive spectroscope (EDS), and attenuated total reflection-fourier transform infrared (ATR-FTIR), respectively. The *in vitro* release study of BMP2 from the Ti was examined in phosphate buffered saline solution (pH 7.4) at 37^{o}C. For the study of osteogenic activity, the 7F2 osteoblast cells were seeded on the Ti samples at a concentration of $1x10^4$ cells/well in an alpha-minimum essential medium (α-MEM). Cell attachment assay was performed by the visual observation of cells using FE-SEM. Incorporated BMP2 on the surface of Ti was determined by anti-BMP2, Goat-anti-rabbit IgG/alkaline phosphatase, using p-nitrophenyl phosphate as a substrate. Alkaline phosphatase (ALP) activity was also evaluated using W-20-17 cell line (ATCC), sensitive to BMP2 with an increase of ALP activity.

Results and Discussion: The BMP2-loaded NC was successfully incorporated into the hydroxyapatite layer on titanium as a trigger of osteogenic differentiation. The incorporated BMP2-loaded NC on the surface of Ti was observed on the surface of HA-coated Ti, marked in black arrow (Fig. 1a). Meanwhile, when the release pattern of BMP2 from the BMP NC-incorporated substrate was examined, the growth factor was continuously released for up to 30 days (Fig. 1b). For *in vitro* study, when the osteoblast cells were cultured for 3 days, they were more proliferating on the BMP2 NC-incorporated substrate than on Ti and HA-coated substrate (Fig. 2a). ALP activity significantly increased as compared with any other groups after 7 and 14 days, respectively (Fig. 2b).

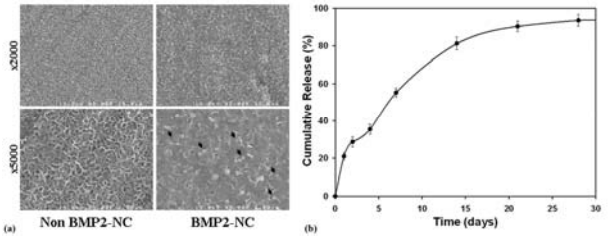

Fig. 1. Characterization of the BMP2 NC-incorporated surface: SEM images of BMP2-NC (black arrow) (a), and *in vitro* release profile of BMP2 from Ti substrate (b).

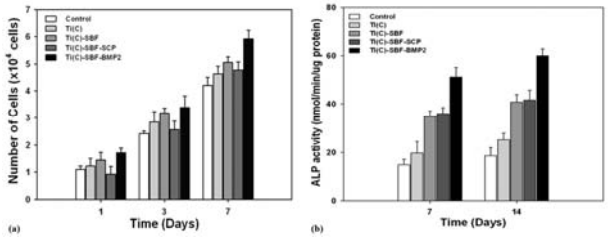

Fig. 2. *In vitro* cell study on the surface of BMP2 NC-incorporated Ti: Cell proliferation using WST-8 (a) and ALP activity per unit protein (b).

Conclusions: In this study, the released BMP2 from BMP2 NC-incorporated Ti substrate can enhance the osteogenic activity of osteoblasts and may facilitate the promotion of osteointegration of a titanium implant with the surrounding bone tissue.

References: 1. Y. Liu et al. *J. Dent. Res.* 2007;86(1):84-89. 2. J.H. Ni et al. *Mater. Res. Bull.* 2008;43:45-53.

Acknowledgment: This work was supported by the grant 2M24210 (KIST) from the Ministry of Knowledge Economy, (K0006028), KOREA.