In vitro evaluation of a chitosan/β-GP gel for a local delivery of BMP-2 for osteoblatic differentiation

Sungwoo Kim, Y Kang, Y Yang
[1]Houston Biomaterials Research Center, Department of Restorative Dentistry and Biomaterials, University of Texas Health Science Center at Houston, Houston, TX 77030

Statement of Purpose: In orthopedic applications, bone morphogenetic protein-2 (BMP-2) is one of the most potent growth factors approved by the FDA for complete bone morphogenesis (e.g., bone remodeling and bone formation).Recent studies have shown the significance of combining biomaterials with BMP-2 in the field of bone regeneration. Encapsulation of BMP-2 in the material structures protects its bioactivity for a longer time period and stimulates proper cellular responses [1]. In this study, a chitosan polymer was used as BMP-2 carrier while a β-glycerophosphate (β-GP) was employed to induce a thermo-sensitive sol-gel transition. The β-GP has also been known as a source of organic phosphate for mineralization and used for osteogenic differentiation [2]. We have focused on investigating the effects of BMP-2 treatment on the behaviors of two cell lines, preosteoblast mouse bone marrow stromal cells (W-20-17) and human embryonic palatal mesenchymal cells (HEPM). The objective of this study was to evaluate the bioactivity of the two cell lines in terms of alkaline phosphatase (ALP) activity, calcium mineral deposition, and osteocalcin synthesis. We also investigated the effect of the Pi released from the chitosan/β-GP gels on cell differentiation and mineralization *in vitro*.

Methods: A 1.5 % (w/v) chitosan solution (≥ 310 kDa, ≥ 75% DDA) was prepared in 0.75% (v/v) aqueous acetic acid and dialyzed at room temperature against distilled water at room temperature (final pH 6.3). BMP-2 was encapsulated into the dialyzed chitosan solutions prior to addition of β-GP (80mM). *In vitro* release behaviors of BMP-2 and Pi from the chitosan gels were measured as a function of time for 3 weeks using a BMP-2 ELISA kit and a SensoLyte MG phosphate assay kit, respectively. Viability of W-20-17 cells and HEPM cells cultured with the gels was quantitatively examined by a MTS assay, and viable cells were observed qualitatively using the live/dead viability assay by a microscope. We evaluated the bioactivity of the two cell lines in terms of alkaline phosphatase (ALP) activity, calcium mineral deposition, and osteocalcin synthesis.

Results/Discussion: Figure 1 shows the cumulative release of BMP-2 from the chitosan gels. The chitosan gels containing a higher concentration (50 ng/ml) of BMP-2 released significantly greater amounts compared to the gels containing a lower concentration (5 ng/ml) for 3 weeks. It was also observed that the incorporation of BMP-2 into the gels could prevent an initial burst release and slow down the overall release rate. In addition, the cumulative release of Pi from the gels was 1.8% and total amount was lower than 1mM at 3 weeks. However, it is expected that β-GP will be quickly hydrolyzed into glycerol and phosphate ion in the presence of bone cells, thereby producing higher concentration of phosphate ion [2]. The W-20-17 cells cultured in the gel group expressed higher ALP activity by about 3.6 and 2.1 folds compared with control groups at 14 and 21 days, respectively. This is probably due to the gradual BMP-2 release from the gel which maintained BMP concentrations at sufficient levels over the culture period. On the other hand, in the cultures of HEPM cells, ALP activity did not significantly increase in response to a sustained BMP-2 treatment. However, only the HEPM cells cultured with the gel group experienced extensive mineralization demonstrated by Alizarin Red S. Our study also demonstrated that supplementation of BMP-2 alone could not accumulate the calcium mineral deposition in both cell lines. However, sustained release of organophosphates (β-GP) from the chitosan gels contributed to significantly higher calcium deposition levels in HEPM cells. In addition, W-20-17 cells expressed significantly higher osteocalcin synthesis at 7 days while HEPM cells expressed higher osteocalcin at 7 and 14 days compared to control groups ($p<0.05$).

Conclusions: BMP-2 incorporated into the chitosan gel for a sustained release were able to induce undifferentiated human mesenchymal cells and mouse bone marrow stroma cells to the osteoblastic phenotype over a prolonged period of time. This study also demonstrated that the calcium mineral formation of bone cells was affected by the presence of phosphate ions in a time, concentration, and species dependent manner.

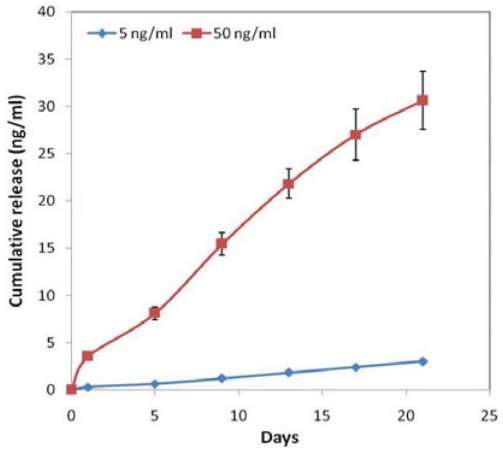

Figure 1. *In vitro* cumulative release profiles of BMP-2 from chitosan gels over a 3-week period. The cumulative release percentage was determined as a function of time by a BMP-2 immunoassay at 450 nm.

References:

1. Diefenderfer DL, Osyczka AM, Garino JP, Leboy PS. J. Bone Joint Surg. Am. 2003a;85A(suppl 2):19-28.

2. Chung CH, Golub EE, Forbes E, Tokuoka T, Shapiro IM. Calcif. Tissue Int. 1992;51:305-11.