Controlled Delivery of BMP-2 from Polyurethane Scaffolds Promotes New Bone Formation in Rat Femoral Defect

Bing Li[1], Scott A. Guelcher[1]
[1]Department of Chemical Engineering, Vanderbilt University

Statement of Purpose: Injection of BMP-2 in a calcium phosphate carrier at one or two weeks after surgery which has a BMP-2 retention period of up to 6 weeks, is more effective than injection within one day to enhance osteotomy-site healing in primates (**Error! Reference source not found.**). Therefore, it is desirable to design a delivery system for sustained release of BMP-2. An injectable polyurethane (PUR) composite scaffold and BMP-2 delivery system is being developed to accomplish this targeted release profile. The strategy of encapsulating BMP-2 in PLGA microspheres was adopted to tune the release profile. By decreasing the size of the PLGA particles, a lower burst and more sustained release is achieved. Through adjusting the release profile of BMP-2 from PUR, the importance of burst and sustained release were evaluated in a rat femoral bone defect.

Methods: PUR scaffolds were synthesized by one-shot reactive liquid molding of hexamethylene diisocyanate trimer (HDIt) with a polyester triol (900-Da) (1). PLGA microspheres were prepared using the double emulsion technique at two average sizes: 80 and 1 μm (3). PLGA microspheres were incorporated into PUR composite scaffolds through mixing with the polyol before the foaming reaction. The internal morphology of the pores in the scaffolds were measured by SEM. *In vitro* release studies were carried out in α-MEM cell culture medium containing 1% BSA at 37 °C. The ALP assay was carried out after incubating MC3T3 osteoprogenitor cells with released BMP-2 for 3 and/or 7 days. For the *in vivo* study, the scaffolds were cut into 3x6 mm cylinders containing 2 μg BMP-2, treated with ethylene oxide gas overnight for sterilization, and implanted into rat femoral defects. The implants were then harvested at weeks 2, 4, and 6 respectively, fixed by formalin, scanned by μCT, decalcified with EDTA treatment, and then processed for histological analysis.

Results: The polyurethane scaffolds are porous and the pores were interconnected as evidenced by SEM imaging. The pore size was ~150 - 600 μm, and the thickness of the pore walls was ~20 μm. BSA-FITC release profiles from PUR scaffolds suggest a lower burst release by adopting the PLGA microsphere encapsulation strategy compared with directly incorporating BSA into PUR scaffold as a powder, and a more sustained release when decreasing the PLGA microsphere size from 80 μm to 1μm (data now shown). Similarly, encapsulation of BMP-2 into large (80 μm) PLGA microspheres decreased the burst release (Figure 1A). The BMP-2 released from PUR scaffolds is bioactive as evidenced by the fact that they enhanced ALP expression by MC3TC osteoprogenitor cells (Figure 1B). After the verification of the bioactivity of released BMP-2 *in vitro*, the cylindrical PUR implants were implanted into rat femoral defects. At week 2, no significant difference between sample and control can be revealed by μCT images, most likely due to the fact that the bone cells are not mineralized yet. However, much more mature bone matrix was visible for the sample treatment groups at week 4 (figure 2). PUR/BMP-2 is the most powerful in terms of promoting new bone formation at the dosage 2 μg BMP-2/implant, with significant formation of new cortex.

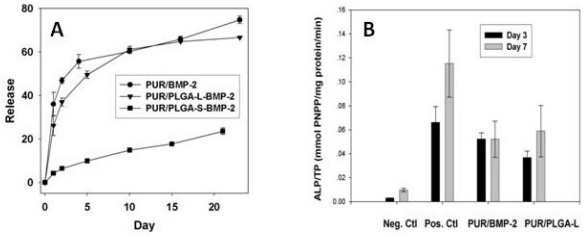

Figure 1. BMP-2 release (A) and *in vitro* bioactivity (B).

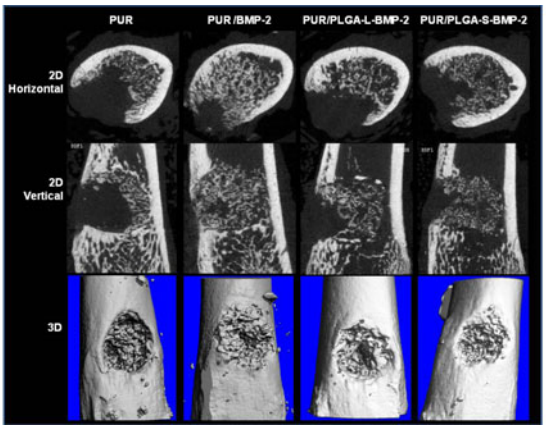

Figure 2. Micro CT images of PUR implants at week 4.

Conclusions: The release of BMP-2 from polyurethane scaffolds is controlled through microencapsulation in PLGA microspheres. The released BMP-2 from PUR scaffolds is bioactive as verified by *in vitro* alkaline phosphatase (ALP) activity assay. Furthermore, the PUR containing BMP-2 powder and/or PLGA microspheres at different sizes demonstrated potential in promoting new bone formation in a rat femoral plug defect. For the femoral plug defect model and selected dosage of 2 μg BMP-2 per implant, PUR containing BMP-2 powder works best which may suggest a critical role of burst release in attacting precursor cells in early stage. However, a lower burst and more sustained release could be essential for critical size defects which take longer time to heal.

References:

1. Seeherman H, Li R, Bouxsein M, Kim H, Li XJ, Smith-Adaline EA, Aiolova M, Wozney JM. J Bone Joint Surg Am. 2006:88:144-160,.
2. Hafeman A. E., Li B., Yoshii T., Zienkiewicz K., Davidson J. M., Guelcher S. A. Pharm Research 2008: [Epub ahead of print],.
3. Utada, A. S., Lorenceau E., Link D. R., Kaplan P. D., Stone H. A., Weitz D. A. Science 2005:308:537- 541.