

# **PLGA Microspheres Embedded in Porous Biodegradable Scaffold as a Delivery Vehicle for Sustained Release of Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2)**

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## **Introduction**

Human recombinant bone morphogenetic protein-2 (rhBMP-2) has been proven effective in stimulating the regeneration of bone in both skeletal and extraskelatal locations. Through encapsulation within, and release from, biodegradable poly(DL-lactic-co-glycolic acid) (PLGA) microspheres the local concentration of rhBMP-2 could be maintained at optimal levels to stimulate bone regeneration [1]. The objective of this work was to investigate the effect of microsphere loading in a porous poly(propylene fumarate) (PPF) scaffold on release characteristics of rhBMP-2.

## **Methods**

PLGA with a 50/50 or 75/25 lactide to glycolide ratio and weight average molecular weight of 25 or 60 kg/mol was used for microsphere preparation using a double emulsion-solvent extraction w/o/w technique. In a test tube, 25 ug of rhBMP-2 in pH 4.5 glutamic acid buffer was added to a solution of 250 mg PLGA in dichloromethane (DCM) with vortexing at 3000 rpm. Next, 2 ml of a 2% poly(vinyl alcohol) (PVA, 88% hydrolyzed, Mw=15 kg/mol) was injected into the test tube and vortexed for an additional 30 s to form the w/o/w double emulsion. The double emulsion was added to 100 ml of 0.3% w/v PVA with stirring. Then, 100 ml of a 2% w/v aqueous isopropanol (IPA) solution was added to extract the DCM and form the microspheres. The microsphere suspension was centrifuged at 2000 rpm, the supernatant phase was removed, washed with distilled deionized (DD) water, lyophilized, and stored at -20°C. To determine the entrapment efficiency, microspheres were dissolved in DCM for 5 min at 37°C and the concentration of rhBMP-2 was measured by enzyme-linked immunosorbent assay (ELISA) using an rhBMP-2 Quantikine kit (R&D Systems, Minneapolis, MN).

PPF was synthesized by a two step polycondensation of diethyl fumarate with 1,2-propanediol as described [2]. The Mw and polydispersity index of PPF was 7.1 kg/mol and 2.3, respectively. Scaffolds were prepared by free radical crosslinking of PPF with 1-vinyl-2-pyrrolidinone (NVP) as the crosslinking agent. Sodium chloride (NaCl) salt crystals with average size of 300 μm were used as the porogen. Benzoyl peroxide (BP) and dimethyltoluidine (DMT) were used as the free radical initiator and accelerator, respectively. 50, 150, and 300 mg of microspheres, corresponding to 5%, 15%, and 30% by weight were added to 1 g of PPF/NVP viscous mixture (0.71 g PPF and 0.29 g NVP). To the mixture, 70% by volume NaCl porogen was added. 50 μl of initiator solution and 20 μl of accelerator solution were added and mixed. The mixture was transferred to a mold and crosslinked for 1 h at 40°C.

To study the release kinetics, a disk was added to each siliconized microvial containing 1 ml of phosphate buffered saline (PBS) with 10% fetal bovine serum (FBS). The microvials were incubated at 37°C with orbital shaking. At each time point, each microvial was centrifuged at 3000 rpm, the supernatant was collected for analysis, and each microvial was

replenished with 1 ml of PBS with 10% FBS. The concentration of rhBMP-2 in the supernatant phase was measured by ELISA.

### Results

The degradation rate of the microspheres, measured by the loss of molecular weight, was highest for 50/50 60kD PLGA sample followed by 75/25 62kD, 50/50 24kD, and 75/25 24kD and it was consistent with the predicted profiles. The first and second numbers for the PLGA type, shown on the x-axis, are for lactide ratio in the copolymer and the copolymer number average molecular weight (Mn), respectively. For example, 50-24 PLGA indicates PLGA with 50% lactide to glycolide ratio and Mn of 24 kD. Addition of 10% FBS to PBS did not have a significant effect on the degradation profile of PLGA microspheres. The encapsulation efficiency depended on BMP-2 concentration, as shown in Figure 1. The encapsulation efficiency for 1 and 4 ug/ml BMP-2 concentration was 20±10% and 60±20%, respectively.

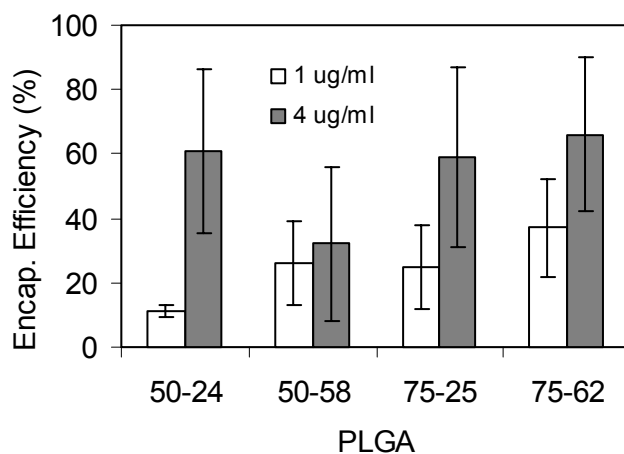


Figure 1

The release profile of rhBMP-2 from PLGA microspheres with 4 ug/ml loading is shown in Figure 2. The burst release was dependent on PLGA molecular weight and lactide to glycolide (L/G) ratio. For example, PLGA with Mn of 24 Kg/mol and L/G ratio of 1 had the lowest burst release of 20 ng/ml and PLGA with Mn of 62 Kg/mol and L/G ratio of 3 had the highest burst release of 140 ng/ml.

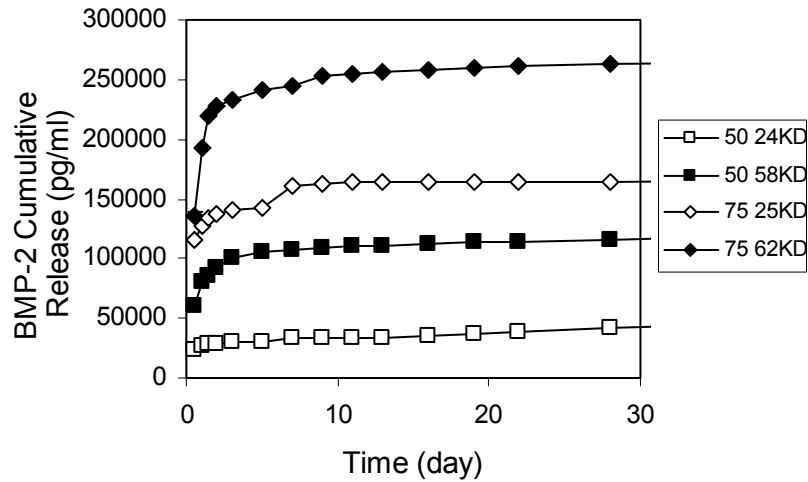


Figure 2

The cumulative amount released after 30 was dependent on the PLGA molecular weight and L/G ratio. PLGA with molecular weight of 62 Kg/mol and L/G ratio of 3 (75/25 62kD) provided the longest sustained release and the highest encapsulation efficiency. The burst release of rhBMP-2 was significantly reduced by embedding the microspheres in porous PPF matrix, as shown in Figure 3. The kinetics of release depended on the fraction of PLGA microspheres in the scaffold. Results demonstrate that embedding PLGA microspheres in a porous PPF scaffold can eliminate the burst release of rhBMP-2 and the released amount can be controlled by the microsphere loading.

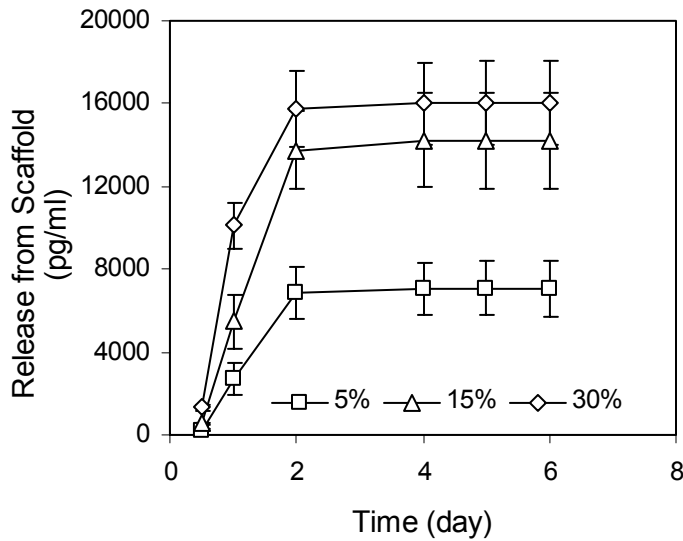


Figure 3

### Conclusions

Results indicate that embedding the microspheres in a porous PPF scaffold can significantly reduce the burst release of rhBMP-2.

### Acknowledgements

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### **References**

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