Sustained In Situ Delivery of rhBMP-2 by Conjugation to Novel Biodegradable Nanoparticles

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Statement of Purpose: When *recombinant human* bone morphogenetic protein (rhBMP-2) is administered as a solution or delivered in a collagen or PLGA sponge, a large fraction of the protein is lost in the process of irrigating the wound, by the action of antibiotics in the first 24 h after the surgical placement, and by soft tissue compression. Furthermore, the use of injectable scaffolds along with minimally invasive endoscopic techniques, now in clinical use, requires rhBMP-2 stabilization against denaturation during injection and in situ hardening [1]. Our laboratory has developed novel bioresorbable succinimide-terminated poly(lactide-co-glycolide fumarate) macromers (PLGF-NHS) that self-assemble into NPs. The objective of this work was to determine the release characteristics of rhBMP-2 conjugated to PLGF-NHS NPs.

Methods: Poly(lactide fumarate) PLAF, PLGF, and poly(lactide-co-ethylene oxide fumarate) (PLEOF) were synthesized by condensation polymerization [2]. Succinimide-terminated macromers (PLAF-NHS or PLGF-NHS) were produced by reacting the hydroxyl end-groups of the PLAF or PLGF macromers with the carbonate group of N,N'-Disuccinimidyl carbonate (DSC). The reaction was allowed to continue for 8 h at ambient conditions. The resulting mixture was precipitated in ether and the product was separated by filtration. The product was purified by precipitation in ether characterized by ^{1}H-NMR.

PLAF-NHS and PLGF-NHS NPs were produced by dialysis of the macromers in dimethylsulfoxide (DMSO)/ N, N-dimethylformamide (DMF) against water with amphiphilic PLEOF macromer used as surfactant to stabilize the NPs. The morphology and size distribution of the NPs was examined with ESEM. The size distribution of NPs was measured by dynamic light scattering. Degradation of the NPs was followed by measuring their mass as a function of incubation time. For conjugation, rhBMP-2 (1 µg/ml in PBS) was allowed to react with the succinimide terminated NPs under ambient conditions for 12 h. After reaction, the suspension was dialyzed against PBS to remove the by-product, N-hydroxy succinimide. For determination of release kinetics, 1 mg rhBMP-2 loaded NPs were incubated with 1 ml PBS (pH 7.4) at 37°C with orbital shaking. At each time interval, the suspension was centrifuged at 15,000 rpm, and the supernatant was removed for analysis. The enzymatically active concentration of the BMP released from the microspheres was measured by enzyme-linked immunosorbent assay (ELISA) using the BMP Quantikine kit (R&D Systems).

Results: The size distribution of PLAF-NHS and PLGF-NHS NPs (90% PLAF-NHS or PLGF-NHS and 10% PLEOF) are compared in Figure 1. The average size of PLAF-NHS and PLGF-MHS NPs was 325 and 250 nm, respectively. PLAF-NHS and PLGF-NHS NPs had similar morphologies, as shown in the insert SEM images.

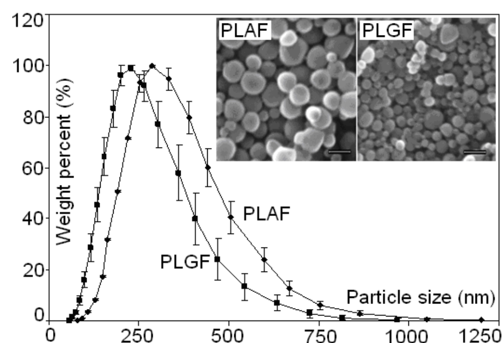

Fig 1. Size distribution of PLAF-NHS and PLGF-NHS NPs.

The conjugation efficiency was determined by measuring the amount of free rhBMP-2 after the conjugation reaction. Conjugation efficiency was >95% for PLAF-NHS and PLGF-NHS NPs. The release of enzymatically active rhBMP-2 from PLAF-NHS and PLGF-NHS NPs is shown in Figure 2. The release of rhBMP-2 from PLAF-NHS NPs was linear with time and >0.4 µg was released after 2 weeks; the release from PLGF-NHS NPs was non-linear and >0.2 µg was released after 2 weeks. PLGF-NHS and PLAF-NHS NPs completely degraded in 25 and 38 days which demonstrated that the release was dominated by erosion of the matrix.

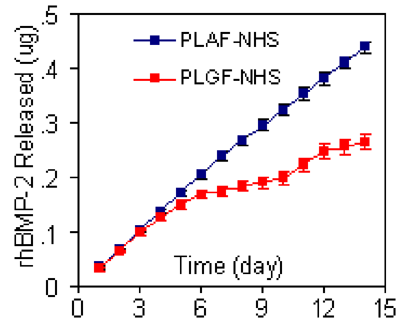

Fig 2. Release kinetics of rhBMP-2 from PLAF-NHS and PLGF-NHS NPs.

Conclusion: Since proteins can be immobilized on PLGF-NHS NPs by the reaction of succinimide groups with amine groups of the protein at physiological condition, conjugated NPs are potentially useful for immobilization and sustained release of differentiation factors like rhBMP-2 in tissue engineering applications.

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References:

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[2] Jabbari E, He X, J. Mater. Sci. Mater. Med. 19(1): 311-318 (2008).