

Preliminary Investigation of Lyophilization To Improve Drug Delivery For Chitosan-Calcium Phosphate Bone Scaffold Construct

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Statement of Purpose: The dual delivery of an antibiotic and growth factor from a composite scaffold would be beneficial for treatment at complex fracture sites such as comminuted fractures and segmental bone defects.

Lyophilized chitosan/calcium phosphate microspheres and scaffolds were fabricated and characterized for drug delivery of growth factors and antibiotics for these applications. Chitosan and calcium phosphate are well-known biomaterials with many attractive features. The aim of this investigation was to increase the loading capacity by taking advantage of the increased porosity due to freeze-drying and to produce an extended elution profile using an additional chitosan bead coating.

Methods: Composite microspheres were fabricated using a method previously described¹. Some spheres were allowed to air-dry and others were lyophilized using a method previously described². Scaffolds were prepared by rinsing hydrated microspheres with a 1 wt. percent acetic acid solution for ten seconds and then placing the adherent spheres into 15 mL centrifuge tubes in which one end has been removed. The scaffolds were then allowed to air-dry for 2-3 days in a chemical fume hood. Some of the scaffolds were rehydrated and lyophilized.

The surface area of scaffolds was obtained with a Micromeritics Gemini V machine using nitrogen gas adsorption. Micro-CT images of scaffolds were obtained with a SkyScan 1076 system, and the porosity of the scaffolds was determined using Skyscan CTanalyzer vs.1.4 imaging software. The compressive moduli of air-dried and lyophilized scaffolds were determined using an Instron load frame. A 5 kN load cell was operated at a rate of 0.5 mm/min. Scaffolds were rehydrated in DI water for four hours before testing.

BMP elutions were performed by loading approximately 100 mg of microspheres with a 5 µg/mL solution of rhBMP-2 at 4° Celsius. An additional chitosan coating was added to some groups of the microspheres as previously described in order to potentially extend the release profile. The microspheres were placed in 2 mL of 1x PBS at 37° Celsius. Samples were taken on days 1, 2, 3, 5, 7, 10, 13, 16, 20, and 26, and the PBS solution was completely refreshed. BMP-2 concentrations were determined using PeproTech BMP-2 ELISA kits.

Results: The freeze-dried scaffolds had considerably more surface area and porosity than the air-dried scaffolds (Table 1). The compressive moduli of air-dried and lyophilized scaffolds were 7.9 ± 1.3 MPa and 10.3 ± 3.4 MPa, respectively. The BMP-2 loading capacity of the microspheres was increased over 25% by lyophilization (Figure 1). An extended BMP-2 elution profile was observed (Figure 2).

The increased porosity brought about by lyophilization provides more surface area for the loading of therapeutic agents such as growth factors like BMP-2 and antibiotics. The efficient local delivery of these

agents can be crucial for the normal healing of complex fractures. The increase in porosity did not cause a decrease in the mechanical stability of the scaffolds. The lyophilized scaffolds did not release more BMP-2 and the supplemental coating did not eliminate the burst effect. However, a desired extended elution in which biologically relevant doses of BMP-2 were still being released after thirty days was observed.

	Surface Area (m ² /g)	Porosity (%)
Air-dried	0.444 ± 0.009	35.8 ± 2.1
Lyophilized	1.935 ± 0.021	53.6 ± 3.6

Table 1: Surface Area and Porosity of Scaffolds

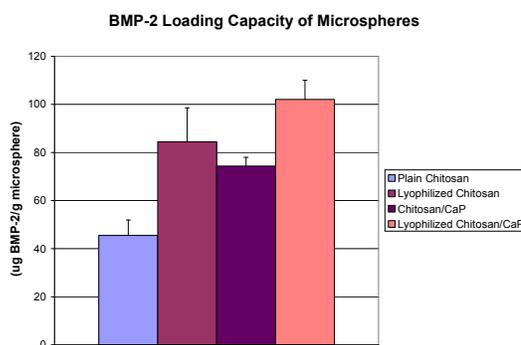


Figure 1. BMP-2 Loading Capacity of Microspheres

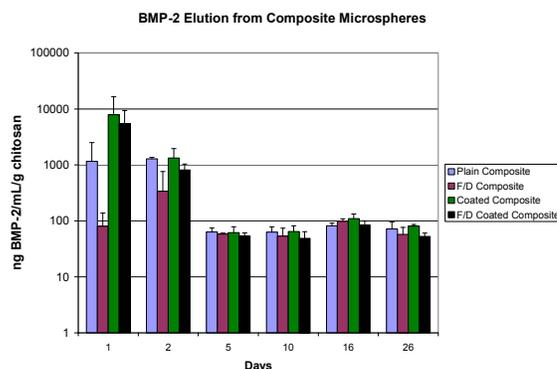


Figure 2. BMP-2 Elution From Composite Microspheres

Conclusions: Lyophilization was found to enhance a number of desired properties of the composite scaffolds. The ability to deliver large amounts of therapeutic agents locally without systemic effects is important clinically in the treatment of complex fractures. This preliminary study encourages the further research of lyophilized chitosan-calcium phosphate scaffolds in bone tissue engineering.

References:

- Chesnutt BM, et al. J Biomed Mater Res A. 2008; Published online 27 Feb.
- Reves, BT, et al. Proceedings of Society for Biomaterials 2006 Annual Meeting, Chicago, IL.