Increased Drug Loading of Composite Chitosan/Calcium Phosphate Microspheres By Lyophilization

B. Reves, G. Utturkar, K. Richelsoph, Y. Yuan, J.D. Bumgardner, W.O. Haggard
Univ. of Memphis

Statement of Purpose: Previous work at our laboratory has produced composite chitosan/calcium phosphate microspheres suitable as a bone graft material[1]. Chitosan was chosen due to its biocompatibility, antimicrobial characteristics, wound healing properties, and ability to stimulate bone formation[2]. Calcium phosphate was chosen due to its osteconductivity[3]. These microspheres have the potential to be loaded with growth factors such as bone morphogenetic protein (BMP) and placed at the site of a bone defect. Thus far, the amount of growth factor able to be loaded into the composite microspheres has been less than optimal. Lyophilization is known to increase porosity. This increased porosity should provide more surface area for increased drug adsorption. This study examines the effect of lyophilization on the properties of the chitosan/calcium phosphate microspheres, especially drug-loading capacity.

Methods: Composite microspheres were made by the method previously described[1]. Some spheres were allowed to air-dry in a chemical hood. Other spheres were placed in deionized (DI) water in small glass vials. These glass vials were placed in either a -20ºC or -80ºC freezer. Once the pre-freezing process was complete, these composite microspheres were lyophilized for 48 hrs. in a Labconco (Kansas City, MO) freeze-dryer.

The morphology and porosity of the microspheres were examined by scanning electron microscopy (SEM). The densities of the air-dried and lyophilized microspheres were determined. Pre-weighed microspheres were placed into a 1mL pipette partially filled with ethanol. The change in volume was recorded for density determination.

The swelling capacity of the composite microspheres was determined by placing pre-weighed microspheres in 3 mL of DI water at room temperature. After 48 hrs., the wet weight of the microspheres was recorded and the swelling ratio determined.

Alkaline phosphatase (ALP) was used as a model protein in order to determine the loading capacity of the microspheres. Pre-weighed amounts of the air-dried and lyophilized microspheres were placed into 3 mL of a 1 mg/ml solution of ALP (MP Biomedicals, Inc.; Solon, OH) in small glass vials. After 48 hrs, samples of the ALP solutions were acquired. The amount of ALP left in the solution was quantified using an ALP assay kit (Sigma Diagnostics; St. Louis, MO). The absorption of p-nitrophenol was read at 410 nm using a Molecular Devices spectrophotometer (Sunnyvale, CA). The amount of ALP adsorbed onto the microspheres was calculated by taking the difference between the initial 1 mg/mL of ALP in solution and the final ALP concentration of the solution.

Results/Discussion: The physical appearances of the air-dried and lyophilized microspheres were quite different. The air-dried microspheres were small and golden, while the lyophilized microspheres were large and white. SEM analysis revealed that the lyophilized microspheres did have increased porosity compared to the air-dried microspheres.

The densities and swelling ratios of the air-dried and lyophilized microspheres were found to be quite different (Table 1). The lyophilized microspheres had a greatly reduced density, indicative of their increased porosity. The lyophilized microspheres had significantly increased swelling ratios. The increase in porosity and surface area allows more water to be retained; thus, more drug should also be adsorbed as well. The lyophilized microspheres did have increased passive adsorption of ALP (Figure 1).

	Density (g/mL)	Swelling Ratio (%)
Air-dried	2.29 ± 0.12	175 ± 7.0
Pre-frozen at -20 ºC	0.40 ± 0.06	468 ± 13.9
Pre-frozen at -80 ºC	0.37 ± 0.01	432 ± 16.3

Table 1: Density & Swelling of Composite Microspheres

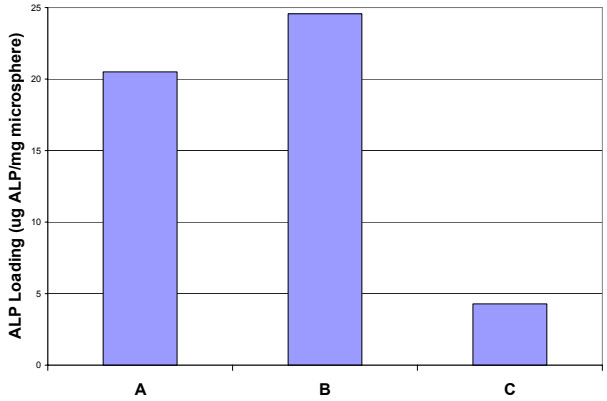

Figure 1: ALP Loading of Composite Microspheres
A: Pre-freezed at -80ºC; B: Pre-freezed at -20ºC; C: Air-dried

Conclusions: Lyophilization of the composite microspheres increased their porosity and swelling capacity and decreased their density. The loading capacity of the spheres with ALP was significantly increased by lyophilization. These results indicate that the lyophilized composite microspheres have the potential to deliver higher amounts of BMP and other growth factors to bone defect sites. An ALP elution study is currently underway, and a BMP loading and elution study will be conducted. Statistical analyses will be performed on all data.

Acknowledgement: Dr. Don Petersen of Biological Innovations for technical assistance.

References:

1. Chesnutt BM, et. al. Proceedings of Society for Biomaterials 2006 Annual Meeting, Pittsburgh, PA.
2. Martino A., et. al. Biomaterials. 2005; 26: 5983-5990.
3. Yang Y., et. al.. Biomaterials. 2005; 26(3): 327-337.