Development of Chitosan-Calcium Phosphate Scaffolds with Increased Degradation for Enhanced Bone Regeneration

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Statement of Purpose: Tissue engineering scaffolds used for bone regeneration should have the following properties: biocompatibility, porosity, adequate mechanical properties, and degradability. The scaffolds must be degradable over an acceptable time frame so that new bone will completely replace the temporary scaffold and normal physiology can be restored. Previously, our labs developed composite chitosan/nano-hydroxyapatite scaffolds that were biocompatible, porous, and exhibited mechanical properties similar to native bone. However, in vitro and in vivo studies have demonstrated that the scaffolds are degrading too slowly and do not allow new bone development in the interior of the scaffolds. The goal of the current research is to alter the fabrication process of the chitosan scaffolds so that the degradation rate of the scaffolds can be optimized. Namely, the following parameters were considered: acid solvent, degree of deacetylation (DDA), chitosan percent (CS%), hydroxyapatite percent (HA%), and neutralization process.

Methods: Composite microspheres were fabricated by dropping a chitosan (Primex ChitoClear®, *Pandalus borealis*) solution containing $NaHPO_4$ and $CaCl_2$ in an acid solvent into a basic precipitating solution (sodium hydroxide, water, and methanol). Glycerol was added to some of the chitosan solutions to act as a thickening agent to prevent the microspheres from breaking apart when dropped into the precipitating solution at lower CS%. After allowing hydroxyapatite to develop by stirring in the precipitating solution for 24 hours, the beads were then neutralized by washing them in deionized water multiple times until a pH of 7.5 was reached. Some of the beads were then placed in a NaAc/HAc buffer at pH 5.0 or 6.5 for approximately twenty minutes.

Table 1 displays the various fabrication parameters for the previous microspheres and the current microspheres.

Table 1: Composite Microsphere Fabrication Parameters

Factor	Previous	Current
Acid Solvent (2% v/v)	Acetic	Acetic, Lactic, Formic
DDA	92.3%	61%, 80%
CS% (w/v)	3.5%	3.5, 3, 2.5%
HA%	Ca^{2+}: 0.1M PO_4^-: 0.06M	0x, 1/2x, 1x (previous), 2x
Glycerol	None	Various
Neutralization	pH 7.5	pH 5.0 or 6.5

The fabrication parameters were altered one at a time so that any change in degradation could be attributed to that single factor.

The amount of degradation was determined using a weight change method. Pre-weighed microspheres were placed in 4 mL of 100 ug/mL lysozyme solution (n=4 samples for each group tested). The solution was refreshed every three days. The microspheres were reweighed after one month, and the percentage of degradation was calculated.

Results: Table 2 displays the results of the degradation study.

Table 2: Degradation of Composite Microspheres

Factor	Effect on Degradation
Acid Solvent	No effect
DDA	**61% DDA has increased degradation**
CS%	Increased degradation but beads rupture as CS% decreases
HA%	No effect
Glycerol	No effect
Neutralization	**Neutralizing to pH ≤ 6.5 greatly increases degradation**

The amount of degradation for beads with 61% DDA was 11.8 ± 0.4% compared to 1.6 ± 0.2% for beads with 80% DDA. Beads neutralized to pH=6.5 and 5.0 displayed degradations of 28.4 ± 0.7 and 32.3 ± 0.5%, respectively; whereas, beads neutralized to pH=7.5 had 14.3 ± 0.5% degradation.

Discussion and Conclusions: Of the factors considered, the DDA of the chitosan and the neutralization process had an effect on the degradation rate of the composite microspheres. As the DDA of chitosan approaches 50%, chitosan degrades more quickly due to the ability of lysozyme to access and enzymatically cleave the glycosidic bonds between the chitosan monomers. Although the 61% DDA microspheres displayed increased degradation, the degradation rate needed to be increased even more. By altering the neutralization process, the degradation rate of the microspheres increased considerably. The approximate 30% degradation over one month should be much more conducive to bone ingrowth into the scaffolds. The pK_a of the amine group in chitosan is 6.5; thus, at pHs lower than 6.5, the amine group will be protonated and chitosan will be positively charged. This positive charge makes chitosan more soluble in an aqueous environment.

Scaffolds can be prepared by washing the microspheres in a dilute acid solution, which causes them to adhere together. Future studies will determine the mechanical and biological properties of the scaffolds made from microspheres with increased degradation. Gel permeation chromatography will also be performed to determine the molecular weight change during degradation. For a bone tissue engineering scaffold to be successful, a delicate balance between degradation and mechanical stability must be achieved.