Scale up and optimization of hybrid microparticles for bone regeneration

A. Champa Jayasuriya, Archana Bhat
Department of Orthopaedics, University of Toledo, Toledo, OH 43614.

Statement of purpose:

The fabrication of a suitable material to use as a scaffold has been a major challenge in the field of bone tissue engineering. Numerous studies have been reported on fabrication of three-dimensional (3D) scaffolds from synthetic polymers like Polyglycolic acid, Polylactic acid, Poly (D, L-lactide-co-glycolide) and natural materials like collagen and chitosan (CS) [1,2]. CS scaffolds have been previously demonstrated the formation of bone *in vivo* [2]. In this study we focus on fabrication of hybrid microparticles (MPs) based on CS. The advantage of using MPs for bone regeneration is that it can be easily injected into the defect site using minimally invasive techniques. In this study we also focus on scale up of the fabrication technique used to make MPs. Scale up of the parameters is important to obtain higher yield of particles without compromising on their characteristics.

Methods:

In this study 1X batches of CS MPs have been fabricated with modification to the emulsification technique previuosly described [3]. 1.5% (w/v) CS solution was prepared by mixing 0.75 gm of CS in 50 ml of 1% (v/v) acetic acid. We have synthesized 1X batches of MPs using 1.5% (w/v) CS solution and then cross-linked with 0, 8, 32, 64, or 110% (w/w) of Tri-polyphosphate (TPP). The different formulations were then scaled up to 4X by mixing 25 ml of CS solution with equal volume of acetone. 36 ml of the mixture was then added drop wise into 600 ml cottonseed oil mixed with 4 ml of span 85. The mixture was stirred for 14 hrs at 37 $^{\circ}$C and an agitation speed of 870 rpm. Then different TPP amount as described above was mixed with 4 ml dH_2O and added to the reaction mixture. 4 hrs after the addition of TPP, equal volume of hexane was added to the mixture. The mixture was vacuum filtered and dried.

We have characterized the MPs using Fourier transformed infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).

Results and Discussion:

Table 1: Comparison of the nature of MPs fabricated using 1.5% (w/v) CS in 1X and 4X formulations.

TPP (%)	Nature of Particles		Yield Ratio	
	1 X	4 X	1 X	4 X
0%	Spherical	no Yield	0.6	N/A
8%	Flaky	Gel	0.55	N/A
32%	Flaky	Flaky	0.65	0.86
64%	Spherical	Spherical	0.68	0.7
110%	Aggregated	not Consistent	0.6	0.65

The 1X batches with 0% TPP gave spherical particles. However, when this formulation was scaled up to 4X, no yield of MPs was obtained after vacuum filtration. This suggested a need for higher cross-linking densities in 4X batches. Optimization on the amount of TPP in 4X batches suggested that the 64% TPP batches gave good particle integrity with fairly good yield ratio.

SEM images have shown that the MPs are spherical in shape with a diameter range of 20-40 µm (data not shown).

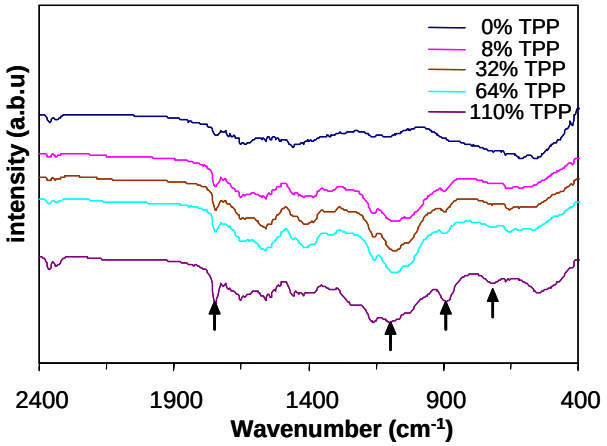

Fig. 1. FTIR spectra of the MPs cross-linked with different TPP amounts.

FTIR analysis of the MPs was useful in determining the chemical structure and the cross-linking behavior of the different batches. FTIR analysis of all samples was performed using the 4X batch except for 0% TPP sample which was performed using the 1X batch, since no yield was obtained in the 4X batch. FTIR spectrum for CS has a characteristic peak for amide I at 1655 cm^{-1} [4]. The characteristic band for the amide I at 1655 cm^{-1} disappears, and a new peak at 1751 cm^{-1} appears, demonstrating the presence of cross-linked MPs. The spectrum for the cross-linked samples showed the characteristic phosphate peaks at 740 cm^{-1}, 910 cm^{-1} and 1107cm^{-1}. The intensity of the new peak at 1751 cm^{-1} and the characteristic phosphate peaks was increased with the increase of TPP amount. This result suggested that the MPs increase cross-linking density with increase of TPP amount.

Conclusion:

The earlier studies in fabrication of CS MPs have involved optimization of 1X batches which gives very low yield. We have successfully scaled up our formulation to 4X batches maintaining high structural integrity. Therefore, these hybrid MPs can be used in bone tissue engineering applications.

References:

1. Langer RJ et al; J. Biomed. Mat. Res., 1993, 27, 183-189.
2. Lee YJ et al; J. Con. Relea. 2002, 78, 187-197.
3. Lopez C et al; Int . J. Pharm. 2006, 312,166-173.
4. Bhumkar DR. et al , AAPS PharmsciTech, 2006, 7 (2).

Acknowledgments:

This study was supported by NSF grant number 0652024.