Development of hybrid microparticles for bone regeneration

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Statement of Purpose: Polymeric microparticles (MPs) have been studied as delivery vehicles for drugs [1], proteins and genes [2]. Recently, MPs were investigated as injectable scaffolds for cartilage tissue regeneration [3]. MPs can be seeded with bone marrow stromal cells (BMSCs) before implantation to function as cell carriers. The main advantage of this microparticle approach, compared with the conventional block scaffolds, is that small particles can be combined with a vehicle and be administered by injection, thus giving the possibility of filling defects of different shapes and sizes through minimally invasive surgery. Upon implantation, the MP-vehicle system is expected to easily conform to the irregular bone defect. Spherical shape of MPs has the flexibility of filling different irregular geometric cavities with closer packing than nonspherical shaped substitutes. We have fabricated and characterized hybrid MPs using chitosan (CS) and calcium phosphate ($CaHPO_4$) that provides necessary osseoconductivity and structural integrity necessary for bone tissue regeneration.

Methods: The shrimp shell CS (85% deacetylated) was purchased from Sigma Chemical Co. (Milwaukee). The hybrid MPs was prepared by modifying the methods previously described [5]. Ionic cross-linking of the CS in the oily suspension medium was achieved by addition of tri-polyphosphate (TPP) solution in water (1 ml, pH 8.5 ± 0.1) in concentrations corresponding to 16% of the amount of CS (w/w). After 4 h of cross-linking, the MPs was isolated by vacuum filtration, washed with an equal volume of *n*-hexane, and freeze-dried. These hybrid MPs were characterized for physical and chemical structure using standard analytical techniques. Murine BMSCs were seeded with CS/$CaHPO_4$ MPs (20 mg) at $5x10^5$ cells per well in a 24-well plates containing standard cell-culture media for 7 days at 37°C.

Results/Discussion: The hybrid CS/$CaHPO_4$ MPs were fabricated using modified double emulsification method confirmed the spherical shape (Fig. 1).

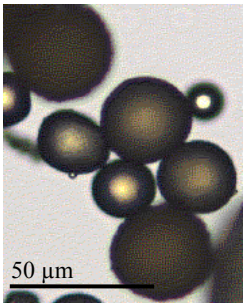

Fig. 1: Light microscope image of hybrid MPs

In order to study the chemical and physical structure of hybrid MPs, FTIR and XRD were performed (Figs. 2 and 3). IR spectra exhibit the as-received CS not cross- linked with TPP, CS MPs cross-linked with TPP, and CS/$CaHPO_4$ MPs cross-linked with TPP. For the CS sample, Amide I and Amide II bands appeared at 1648 and 1580 cm^{-1}, respectively. These amide bands were shifted to 1668 and 1542 cm^{-1} after cross-linking CS with TPP. The cross-linked CS also showed new peaks at 1741 and 1154 cm^{-1} due to the linkage between TPP groups and ammonium ions in the CS. Similar results were reported when CS nanoparticles or films were prepared by cross-linking with TPP [4]. In addition, the characteristic phosphate bands at 1037 and 563 cm^{-1} appeared for both types of MPs, confirming the presence of phosphate groups. The CS stock sample exhibited the main peak at 2θ =20º in XRD. This peak was observed in the same region of XRD due to the existence of CS in CS/$CaHPO_4$ MPs (Fig. 3). This XRD patterns also exhibited sharp characteristic peaks, confirming the presence of TPP and $CaHPO_4$ in the CS MPs.

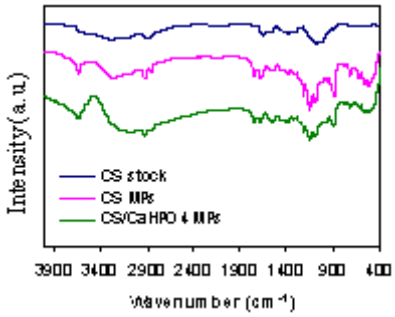

Fig. 2: FTIR spectra of CS stock, CS MPs cross-linked with TPP, CS/$CaHPO_4$ MPs cross-linked with TPP.

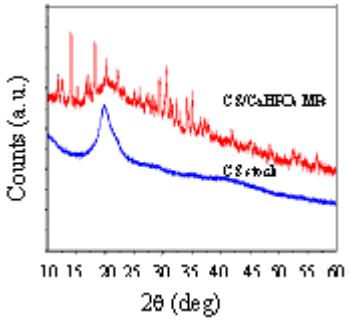

Fig. 3: X-ray diffraction (XRD) spectrum of CS stock, CS/$CaHPO_4$ MPs (baseline was shifted).

Conclusions: These hybrid MPs have shown the characteristics of both CS and $CaHPO_4$. These MPs provides osteoconduction and unique properties of CS which are necessary for bone regeneration. The spherical shape of MPs is important feature for MPs since they can easily fill the irregular shaped bone defects. These MPs were shown the capability of BMSCs culture with osteogenic media up to 7 days according to our preliminary data.

References: [1]. Varde NK, et al., Expert Opin Biol Ther. 4(1):35-51. 2004. [2]. Frenkel PA, et al., Ultrasound Med Biol. 2002, 28(6):817-822. [3]. Thissen H, et al., J Biomed Mater Res A. 2006, 77(3):590-598. [4]. Bhumkar DR, et al., AAPS PharmSciTech 7(2) Article 50, 2006.