PLAGA/Nanocrystal Hydroxyapatite Composite Scaffolds for Bioreactor Based Bone Tissue Engineering

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Statement of purpose: Bone tissue engineering has emerged as an alternative approach to conventional bone grafting methods. One concern in bone tissue engineering is the limited bone ingrowth into scaffolds due to inadequate diffusion of oxygen, nutrient, and waste. We have reported a dynamic flow culturing system using high-aspect-ratio vessel (HARV) rotating bioreactor and 3-dimensional (3D) scaffolds with mixed lighter-than-water (LTW) and heavier-than-water (HTW) 85:15 Poly glycolide-co-lactide (PLAGA) microspheres[1]. Nanophase ceramics have been shown to enhance the function of osteoblast cells when added to PLAGA to form composite scaffolds[2]. To incorporate the advantage of nanophase ceramics, the aim of current study is to develop LTW and HTW PLAGA/nanocrystal hydroxyapatite (n-HA) composite microspheres and sinter them to form mixed scaffolds suitable for use in a HARV bioreactor.

Materials and Methods: LTW and HTW microspheres were fabricated using solvent evaporation technique. Briefly, n-HA (average diameter 100nm, from Berkeley Advanced Biomaterials, Inc.) particles were dispersed in 20%(w/v) PLAGA (Mw=110kDa)-methylene chloride solution, which was then poured into 0.1% (for LTW) or 1% (for HTW) Polyvinyl alcohol solution and stirred at 1200 rpm (for LTW) or 250 rpm (for HTW) for 24 hours. The actual concentration of n-HA in the microspheres was determined by thermogravimetry analysis (TGA) and the surface morphology was examined by Scanning Electron Microscope (SEM) (Jeol 6700, Japan). Mixed scaffolds were fabricated using the sintered microsphere technique developed in our laboratory[3]. LTW and HTW microspheres of diameter between 600 ~710µm were packed in a 50:50 (w/w) ratio in a stainless steel mold and heated above the glass transition temperature of PLAGA. Compressive tests on the scaffolds (5mm×10mm) were performed using an Instron 5544 mechanical tester. A Micromeritics Autopore II mercury porosimeter was used to determine the pore size and density of the scaffolds. Statistical analysis was done using ANOVA/Tukey test ($p<0.05$).

Results and Discussion: The results of TGA are summarized in Table 1. The data show that n-HA is successfully incorporated into microspheres. SEM images (not shown) reveal the presence of well-dispersed n-HA particles on the surface of both LTW and HTW microspheres. The effects of sintering temperature and time on the mechanical properties of 4 different PLAGA/n-HA ratio scaffolds were investigated by sintering them at different temperatures for different time periods. Figure 1 shows the effect of sintering time and temperature on the mechanical properties of PLAGA/n-HA 4:1 ratio scaffolds. The compressive modulus of scaffolds sintered at 90°C for 3 hours were found to be significantly higher than other sintering conditions. Sintering at higher temperatures and longer time periods both enhances the extent of fusion between the microspheres and hence the mechanical properties. However, shrinkage of LTW microspheres at longer sintering time resulted in decreased mechanical properties (Fig. 1: 90°C 4h). The highest compressive modulus of each composition of scaffolds were found and compared in Fig. 2 to show the effect of n-HA concentration on the mechanical properties of them. The compressive modulus of PLAGA/n-HA 4:1 scaffold is significantly higher than all the other compositions studied. This is probably due to the optimal distribution of n-HA on the surface of microspheres. At higher concentration of n-HA such as PLAGA/n-HA 1:1, the SEM (not shown) revealed high coverage of n-HA on the surface, which adversely affected the sintering process resulting in significantly lower mechanical properties. The porosimetry results show that PLAGA/n-HA 4:1 scaffold has a median pore diameter of 197.03±2.80µm and a bulk density of 0.7419±0.0274g/ml when sintered at 90°C for 3 hours. The density of scaffold is less than the media density (1g/ml), which enables the scaffolds to have proper trajectories in HARV bioreactors without hitting the wall.

Table 1. Actual HA content in different microspheres by TGA.

Sphere type	PLAGA/HA initial ratio			
	1:1	2:1	4:1	10:1
LTW	46.60%	29.75%	18.10%	10.63%
HTW	34.60%	30.01%	19.15%	9.06%

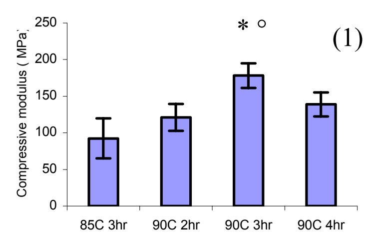

Fig 1 Mechanical properties of PLAGA/HA 4 to 1 scaffold at different sintering temperature and time. (*) and (°) indicate significant difference comparing different sintering temperature and time.

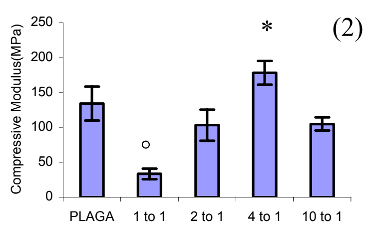

Fig 2 Mechanical properties of different compositions of scaffolds. (*) indicates significantly higher than all the others. (°) indicates significantly lower than all the others.

Conclusions: We demonstrated the feasibility of developing PLAGA/n-HA composite matrices as scaffolds for bioreactor based bone tissue engineering with suitable porous structure and bulk density. The composite scaffolds with PLAGA/n-HA ratio 4:1 show the highest compressive modulus when sintered 90°C for 3 hours.

References: 1. Yu X., et al. PNAS 2004; 101: 11203-8

2. Webster T, et al. JBMR A 2005; 74(4):677-86
3. Borden M., et al. Biomaterials 2003; 24: 557-609