# Nano-hydroxyapatite/Polyhydroxybutyrate-co-valerate Composite Scaffolds for Bone Tissue Engineering

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**Introduction**

Biodegradable polymers are normally used for tissue engineering (TE) scaffolds as they can lead to the formation of a completely natural tissue without leaving permanently synthetic element(s) in the human body. Polymers such as polyhydroxybutyrate-co-valerate (PHBV) have been used to construct TE scaffolds (Sultana N and Wang M, Key Engg Mater, 2006, in press). Different application situations require scaffolds of different characteristics. In order to seek better scaffolds for bone tissue engineering, a composite strategy can be adopted (Wang M, Biomaterials, 2003; 24:2133-2151). In this paper, the fabrication and characterization of nano-hydroxyapatite/PHBV composite scaffolds are reported.

**Materials and Methods**

For the fabrication of hydroxyapatite (HA)/PHBV composite scaffolds, the previously established emulsion-freezing / freeze-drying technique for producing pure PHBV scaffolds was adopted. However, modifications were made to this technique in order to incorporate HA nanospheres. The HA nanospheres were produced using a nanoemulsion method (Zhou WY, et al., J Mater Sci-Mater M. 2006; in press). In producing the composite scaffolds, chloroform and acetic acid were used as the solvent and the water phase, respectively. Typically, 10 ml of PHBV emulsion was prepared and an amount of nano-HA was dispersed in the emulsion in a beaker. The beaker containing the mixture was put in a freezer overnight to solidify the emulsion and then freeze-dried for at least 48 hrs to remove the solvent and water phase completely. The skeletal density and porosity of composite scaffolds were measured. The porous structures of scaffolds were studied using scanning electron microscopy (SEM). The crystallinity of polymer matrix was investigated using differential scanning calorimetry (DSC). Compressive mechanical properties of scaffolds were determined using a mechanical tester (Instron 5848, USA).

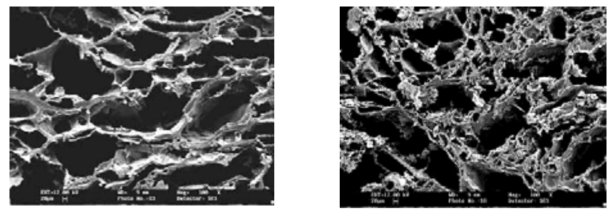
**Results and Discussion**

It was found previously that using optimized polymer solution concentrations, PHBV scaffolds fabricated could possess interconnecting and anisotropic porous networks with >80% porosity and pore sizes of 50-300 µm. In this study, a series of composite scaffolds containing 5 to 30% of nano-HA were fabricated using emulsions of polymer solution concentrations of 5, 7.5 and 10% w/v PHBV. The density of composite scaffolds was found to increase while the degree of crystallinity and porosity decreased with an increasing amount of nano-sized HA in the scaffolds (Table 1).

Good dispersion and good adhesion of HA nanospheres in the PHBV matrix were found to be present. With the incorporation of nano-HA, HA/PHBV composite scaffolds exhibited nearly the same porous morphology as that of plain PHBV scaffolds (Fig.1), indicating the feasibility of the production technology used.

Table 1: Crystallinity ($X_c$), density and porosity of composite scaffolds

| Scaffold (10% w/v PHBV) | $X_c$ (%) | Density ($g/cm^3$) | Porosity (%) |
|---|---|---|---|
| PHBV | 56.88 | 0.1961 | 84 |
| 5% HA/PHBV | 52.87 | 0.2110 | 83 |
| 10% HA/PHBV | 52.17 | 0.2601 | 80 |
| 20% HA/PHBV | 46.28 | 0.3102 | 78 |



(a) PHBV (b) 10% HA/PHBV

Fig.1: SEM micrographs of scaffolds produced using the 7.5% w/v PHBV emulsion

From compression tests, it was found that while PHBV scaffolds had a compressive modulus of 1.8 MPa, 20% HA/PHBV composite scaffolds had the modulus of 4.2 MPa, which is significantly greater ($p<0.05$). Compressive curves of scaffolds produced (Fig.2) exhibited the mechanical behaviour of typical porous solids. Compressive testing also demonstrated mechanical anisotropy of composite scaffolds.

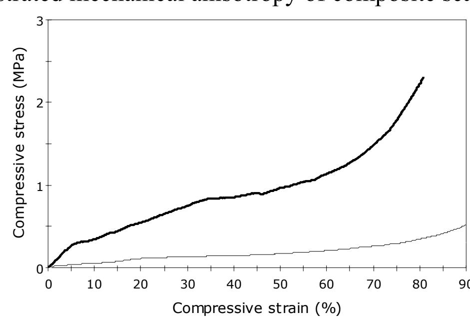



Fig.2: Compressive stress-strain curves of PHBV (lower curve) and 20% HA/PHBV (upper curve) scaffolds

**Conclusions**

Highly porous composite scaffolds could be fabricated using the emulsion-freezing / freeze-drying technique. Composite scaffolds possessed enhanced mechanical properties than pure polymer scaffolds. It is expected that HA/PHBV scaffolds will provide conducive environment for cell attachment and tissue formation in bone tissue engineering as the HA incorporated is osteoconductive.

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