

Fabrication of porous ultra-short single-walled carbon nanotube nanocomposite scaffolds for bone tissue engineering

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Abstract

We investigated the fabrication of highly porous scaffolds made of three different materials [poly(propylene fumarate) (PPF) polymer, an ultra-short single-walled carbon nanotube (US-tube) nanocomposite, and a dodecylated US-tube (F-US-tube) nanocomposite] in order to evaluate the effects of material composition and porosity on scaffold pore structure, mechanical properties, and marrow stromal cell culture. All scaffolds were produced by a thermal-crosslinking particulate-leaching technique at specific porogen contents of 75, 80, 85, and 90 vol%. Scanning electron microscopy, microcomputed tomography, and mercury intrusion porosimetry were used to analyze the pore structures of scaffolds. The porogen content was found to dictate the porosity of scaffolds. There was no significant difference in porosity, pore size, and interconnectivity among the different materials for the same porogen fraction. Nearly 100% of the pore volume was interconnected through 20 μm or larger connections for all scaffolds. While interconnectivity through larger connections improved with higher porosity, compressive mechanical properties of scaffolds declined at the same time. However, the compressive modulus, offset yield strength, and compressive strength of F-US-tube nanocomposites were higher than or similar to the corresponding properties for the PPF polymer and US-tube nanocomposites for all the porosities examined. As for *in vitro* osteoconductivity, marrow stromal cells demonstrated equally good cell attachment and proliferation on all scaffolds made of different materials at each porosity. These results indicate that functionalized ultra-short single-walled carbon nanotube nanocomposite scaffolds with tunable porosity and mechanical properties hold great promise for bone tissue engineering applications.

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1. Introduction

A scaffold is one of the key components in the tissue engineering paradigm in which it can function as a template to allow new tissue growth and also provide temporary structural support while serving as a delivery vehicle for cells and/or bioactive molecules [1,2]. An ideal scaffold for bone tissue regeneration should possess mechanical properties similar to the bone tissue being

replaced, good biocompatibility with surrounding tissue, large porosity and pore size, high pore interconnectivity for bone tissue ingrowth, and biodegradability such that it is gradually replaced by growing bone tissue [3]. Despite extensive research, no existing man-made scaffold can meet all these requirements. The development of novel biomaterials and scaffold fabrication techniques is critical for the success of bone tissue engineering.

Recently, a variety of nanocomposite materials made of poly(propylene fumarate) (PPF) and single-walled carbon nanotubes (SWNTs) have been explored for potential use as scaffold materials in our laboratory [4–6]. These nanocomposites are injectable, thermally crosslinkable, and cytocompatible *in vitro*, making them promising

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biomaterials for bone tissue engineering. SWNTs, especially ultra short SWNTs (US-tubes), significantly reinforced PPF polymer, whose inferior mechanical properties often limit its use as a highly porous scaffold for load bearing applications. Chemical functionalization of SWNTs can improve their dispersion into PPF, augmenting their reinforcing effects [5]. Therefore, functionalized US-tubes (F-US-tubes) were introduced here to investigate their effects on scaffolds for bone tissue engineering.

In this study, we examined the scaffold fabrication process to generate three different materials: pure PPF polymer, US-tube/PPF nanocomposite, and F-US-tube/PPF nanocomposite. While scaffolds with a high porosity would allow bone tissue ingrowth, their mechanical properties could become compromised [7]. Therefore, scaffolds with porosities of 75, 80, 85, and 90 vol% were designed for this experiment. To our knowledge, few studies have produced a SWNT nanocomposite scaffold for bone tissue engineering and there has been little characterization of such highly porous scaffolds for their physical and biological properties [8–11]. We demonstrate here that up to 90 vol% scaffolds of nanocomposites can be reproducibly created via thermal-crosslinking and salt porogen leaching. Furthermore, we ask the following questions: (1) How do the carbon nanotubes affect the pore structure and mechanical properties of scaffolds? (2) What are the effects of different porosity on the pore structure and mechanical properties of scaffolds? (3) What are the responses of bone marrow stromal cells cultured on scaffolds made of different nanomaterials with varying porosity?

2. Materials and methods

2.1. Polymer preparation

PPF and its crosslinking agent propylene fumarate-diacrylate (PF-DA) were synthesized as previously described [12,13]. The PPF used in this study had a number average molecular weight (M_n) of 2660 and a polydispersity index of 2.1. PF-DA had a molecular weight of 340. All reagents and organic solvents were used as received. PPF and PF-DA were mixed at a 1:2.1 mass ratio prior to usage.

2.2. US-tube synthesis and functionalization

US-tubes were produced by fluorination followed by pyrolysis of as-received SWNTs as previously described [14]. The resulting nanotubes had lengths of 20–80 nm and were much shorter than SWNTs produced by a high pressure carbon monoxide (HiPco) process which are hundreds of nanometers to several microns in length [15]. US-tubes were then functionalized by an alkylation-based reduction [16]. Briefly, NH_3 (~60 mL) was condensed into a flame-dried 100 mL flask loaded with US-tubes (20 mg, 1.7 mmol of carbon). This was followed by the addition of sodium metal (184 mg, 8 mmol), and then 1-iodododecane (1.9 g, 6.4 mmol). The reaction mixture was subsequently allowed to stir overnight with the slow evaporation of NH_3 . The remaining mixture was diluted with ethanol (10 mL) and then with water (20 mL). After acidification with 10% HCl, the US-tubes were extracted into hexane (50 mL), and washed three times with water (50 mL each time). The final hexane layer was filtered through a 0.2 μm PTFE membrane filter, washed

with ethanol (200 mL) and chloroform (200 mL), and then dried to give functionalized US-tubes (F-US-tubes, Fig. 1). The functionalization was confirmed by the large disorder band (at $\sim 1290\text{ cm}^{-1}$) in the Raman spectra of F-US-tubes. The mass loss of F-US-tubes in thermogravimetric analysis (TGA, $10^\circ\text{C}/\text{min}$ to 800°C in argon) was 40% indicating that one out of every 21 carbon atoms on the sidewalls of US-tubes was covalently attached with a dodecyl group [17].

2.3. Fabrication of nanocomposites and their scaffolds

Following an established procedure of high shear mixing, sonicating and drying [5], the carbon nanotubes were uniformly distributed into the PPF/PF-DA mixture (subsequently referred to as PPF). US-tubes were loaded at 0.5 wt% concentration because US-tube/PPF nanocomposites achieved their maximum mechanical properties at this loading concentration [6]. F-US-tubes were loaded at 0.83 wt% concentration to provide the nanocomposite with the same amount of carbon nanotubes as the US-tube/PPF nanocomposite.

Porous scaffolds were fabricated by a thermal-crosslinking particulate-leaching technique with NaCl as the water soluble porogen [11]. PPF or the nanocomposites were first mixed with 1 wt% free-radical initiator, benzoyl peroxide, followed by the addition of the appropriate amount of NaCl (300–500 μm crystal size) sieved with USA Standard Testing Sieves (Fisher Scientific, Pittsburgh, PA). The mixtures were then cast and thermally crosslinked at 100°C for 24 h in cylindrical Teflon molds (4 mm diameter and 8 mm height) or cylindrical glass molds (6.5 mm diameter). The 100°C curing temperature was applied here to ensure complete crosslinking of the scaffold materials [18]. After being removed from the molds, the $4 \times 8\text{ mm}$ samples were used for the characterizations of pore structure and mechanical properties and the 6.5 mm-diameter samples were cut into 2 mm thick discs for cell seeding. Finally, all crosslinked samples were soaked in water (water was changed every 8 h) on a shaker table (80 rpm) at room temperature for 3 days to leach out the NaCl porogen. Afterwards, they were blotted with absorbent paper and then vacuum dried for 24 h.

The amount of NaCl used to generate 75, 80, 85, and 90 vol% porous scaffolds was calculated according to the following equations:

$$\varepsilon = \frac{V_{\text{NaCl}}}{V_{\text{NaCl}} + V_{\text{Nano}}} \times 100\%, \quad (1)$$

$$W_{\text{NaCl}} = \frac{\varepsilon}{1 - \varepsilon} \times \frac{\rho_{\text{NaCl}}}{\rho_{\text{Nano}}} \times W_{\text{Nano}}, \quad (2)$$

where ε is the apparent porosity (volume percent of porogen in a scaffold), V_{NaCl} and V_{Nano} are the volumes of NaCl and the nanocomposite in a scaffold, W_{NaCl} and W_{Nano} are the weights of NaCl and the nanocomposite in a scaffold, and ρ_{NaCl} is the density of NaCl (2.17 g/mL). The density of the nanocomposite (ρ_{Nano}) was calculated by measuring the mass and volume of five solid crosslinked nanocomposite cylinders and found to be 1.25 g/mL. Based on these theoretical calculations, 83.9, 87.4, 90.8, and 94.0 wt% NaCl is needed to achieve 75, 80, 85, and 90 vol% porous scaffolds. For example, a formulation of 1 g nanocomposite mixed with 15.62 g NaCl would yield a 90 vol% scaffold.

2.4. Scanning electron microscopy (SEM)

SEM was applied to examine the pore structure of a scaffold, such as pore size, morphology, and interconnectivity. Cross-sections of cut disc samples were sputter-coated with gold for 2 min at 100 mA using a CrC-150 Sputtering System (Torr International, New Windsor, NY) and observed under a FEI Quanta 400 field emission scanning electron microscope (FEI Company, Hillsboro, OR) at an accelerating voltage of 15 kV.

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