Bioactive glass nanocomposite scaffold toughed by multi-wall carbon nanotubes for tissue engineering

Jinglin LIU,* Chengde GAO,* Pei FENG,* Tao XIAO,∗∗,**,**,** Jun SHUAI∗∗,∗∗,† and Shuping PENG∗∗∗,∗∗∗,‡§

*State Key Laboratory of High Performance Complex Manufacturing, Central South University, Changsha 410083, China
**Orthopedic Biomedical Materials Institute, Central South University, Changsha 410083, China
***Department of Orthopedics, The Second Xiangya Hospital, Central South University, Changsha 410011, China
****Hunan Provincial Tumor Hospital and the Affiliated Tumor Hospital of Xiangya School of Medicine, Central South University, Changsha 410013, China
*****School of Basic Medical Science, Central South University, Changsha 410078, China

Bioactive glasses have wide application prospects in bone replacement and regeneration owing to the unique osteoconductivity and osteostimulativity. However, the high brittleness and poor compressive strength limit their applications in load-bearing positions. In this study, multi-wall carbon nanotubes (CNTs) were used to toughen bioactive glass (13–93 glass), and their nanocomposite scaffolds were fabricated by selective laser sintering. The effect of CNTs amount (1–5 wt.%) on mechanical properties of the scaffolds was investigated. The results showed that CNTs were effective to improve the mechanical properties of the nanocomposite scaffolds by virtue of the toughening mechanisms of bridging, pull-out and crack deflection. The optimum compressive strength and fracture toughness reached to 37.32 MPa and 1.58 MPa·m1/2, respectively, by adding the appropriate amount of CNTs (3 wt.%). In addition, the bone-like hydroxy-carbonate apatite (HCA) layer was formed on the surface of the nanocomposite scaffolds after immersion in simulated body fluid (SBF) for 10 days. And the cell culture test showed that the scaffolds should have good cytocompatibility. The research indicated that the 13–93 glass-CNTs nanocomposites scaffolds are promising candidates for bone tissue engineering applications.

Key-words : Carbon nanotubes, Bioactive glass, Mechanical property, Selective laser sintering

1. Introduction

Bioactive glasses based on silicate systems such as 13–93, 45S5 have a widely recognized ability to produce a bone-like HCA layer, to foster the growth of bone cells, and to bond strongly with hard and soft tissues.1–5 In addition, the release of the ionic dissolution products from bioactive glasses (such as Si, Ca, P and Na) can induce favorable intracellular and extracellular responses and more rapidly promote the new bone formation.6,7 So, the bioactive glasses have been considered as the promising scaffold materials for bone tissue engineering. Among all the bioactive glass, 13–93 glass has been growing attention as the third-generation bioactive material. Compared to the most common used 45S5 glass, 13–93 glass can further accelerate the body’s natural ability to heal itself, its higher amount of SiO2 content can slow down the reaction rates after implantation. Moreover, the low sintering temperature (between 675 and 700°C) in 13–93 glass can provide better viscous properties to sinter the complex shapes.8,9 However, their inherent high brittleness and low strength limit their use to only non-load-bearing applications. Undoubtedly, improving the mechanical properties becomes the focus of research work to bioactive glasses.10,11

CNTs as well-ordered and all-carbon hollow graphitic nano-materials, are attracting much attention because of the extraordinary intrinsic mechanical, electrical, functional and thermal properties.12,13 For example, their exceptional strength (up to 200 GPa), Young’s modulus (up to 1200 GPa) and high aspect ratio (about 1000) make them to be promising toughening phases for the various materials classes to improve the mechanical properties.14 Accordingly, the several nanocomposites have been developed by incorporating CNTs into polymers, metals and ceramics.15–17

Currently, the fabrication techniques of the scaffolds commonly include the pressureless sintering, hot isostatic pressing and reactive hot pressing.18 However, in the above sintering methods, the high temperature and long-time reactive environment damage CNTs and effect their toughening result (CNTs burn completely at 750°C in air).19 Selective laser sintering (SLS), as one of the solid freeform fabrication (SFF) techniques, employs the laser beam to selectively sinter multiple layers of powder to build the three-dimensional part according to a computer-aided design (CAD) model. The technique can fabricate the customized intricate geometries and the controllable pore structure of the scaffolds, which can promote cell ingrowth and nutrients delivery.20–24 Besides, SLS can effectively reduce the damage to CNTs due to quick heating rate and slow sintering period.25

In view of the above analysis, multi-wall CNTs were added into 13–93 glass to improve the mechanical properties. The nanocomposites scaffolds were fabricated by selective laser sintering (SLS). The influence of CNTs on the mechanical properties (including fracture toughness, Vickers hardness, compressive strength and Young’s modulus) and their toughening mechanism were investigated. In addition, the bioactivity and the cell adhesion of the scaffolds were evaluated by immersing them in
the simulated body fluid (SBF) for 10 days and culturing the human osteosarcoma MG-63 cells on their surface for 6 days, respectively.

2. Experimental procedure

2.1 Materials and Processing

13–93 glass (the chemical composition of 53% SiO2, 20% CaO, 6% Na2O, 4% P2O5, 5% MgO and 12% K2O in wt.%) was prepared by melting the mixture of analytical grade Na2CO3, K2CO3, MgCO3, CaCO3, SiO2 and NaH2PO4·2H2O in a platinum crucible and quenching between cold stainless steel plates. Then the glass was crushed, ground in a hardened steel mortar and milled for 2 h in an attrition mill with high-purity Y2O3-stabilized ZrO2 milling media and ethanol as the solvent. Finally, 13–93 glass was obtained with the particle size range of about 100 nm.

Multi-wall CNTs were purchased from Nanjing XFNano Material Tech Co., Ltd. The original CNTs were subjected to steam purification at 900°C for 4 h in order to remove the amorphous carbon and other graphite particles. The purified CNTs were then subjected to nitric acid refluxing at 100°C for 24 h in order to remove catalytic metal particles. This was followed by ultrasonication for 2 h in 450 ml of 81:(v:v) NH4OH-ethanol solution for partial esterification of the carboxylic acid groups.25) Finally the CNTs were filtered through a 30 nm polycarbonate membrane and dispersed in ethanol using ultrasonication.

13–93 glass was mixed with the different weight fraction of CNTs (1, 2, 3, 4, and 5 wt.%) respectively by wet methods using high energy ball milling machine in an ethanol environment. In the ball milling process, alcohol was used as dispersed medium to prevent the agglomeration of the CNTs. And then the 13–93 glass-CNTs nanocomposite scaffolds were fabricated via a homemade SLS system. The concrete fabrication processes are as follow:26-28) the nanocomposite powder of about 0.1 mm thickness was laid on the powder bed. And then the powder was selectively sintered according to the scanning path controlled by the computer. The major SLS process parameters included laser power 6 W, scan speed 100 mm/min and laser beam spot size 800 μm. After the first layer was sintered, the powder bed moved down one layer depth and a second layer of powder was spread and sintered until the complete scaffold was sintered. The monolithic 13–93 glass scaffold was also fabricated under the same condition as control.

2.2 Characterization

Indentation experiments were carried out to measure the hardness and fracture toughness of the scaffolds using Vickers hardness tester (TMVS-1, Beijing times Co., China) with the load of 49 N for 30 s. And the crack length was recorded using the indenter microscope. Then, Vickershardness HV can be directly obtained from the tester, and the fracture toughness (KIC) was calculated according to the following equation:29)

$$K_{IC} = 0.0824 \left( \frac{P}{c^{1/2}} \right)$$

Where, P was the indentation load (N) and c was the length of the well-developed median crack.

The compressive strength (σ) and Young’s modulus (E) of the scaffolds were characterized using an uniaxial Instron mechanical tester (WD-D1, Shanghai Zhuoji Instruments Co. China) at ambient temperature in air. Specifically, the testing specimen of the scaffold was the compact cubic structure with the smooth surface, its specific size was 2 mm in length, 1 mm in width and 1 mm in height. First, the testing specimen was vertically mounted on two mechanical gripping units of the tester. Then the scaffold specimen was pressed under the uniaxial strain load with a constant strain rate of 0.5 mm/min until the scaffold specimen ruptured. Finally, compressive strength (σ) and Young’s modulus (E) were calculated based on the following equations:29)

$$\sigma = F/A$$

$$E = KL/A$$

Where, F was the maximum uniaxial strain load; A was the average of surface area, K was the stiffness, L was the length of the tested specimen, and A was the average of surface area. For the above results, five scaffolds specimens were tested to obtain the average on the same condition. In addition, the porosity of the complete scaffold was estimated based on Archimedes' principle.30)

To evaluate the distribution of CNTs in the 13–93 glass, the surface morphologies of the original 13–93 glass, CNTs, nanocomposite powder and the sintered scaffolds specimens with the different amount of CNTs were investigated by scanning electron microscopy (SEM). Besides, to investigate the effect of the amount of CNTs on the mechanical properties and the toughening mechanisms, the fractured surfaces during the crack propagation paths produced by Vickers indentation were observed using SEM. The tested samples were coated with a thin layer of gold (Au) and then were observed on a SEM (JSM-6490LV, JEOL, Japan) at 20 kV, magnification 20–300,000×, resolution <3.0 nm, and 1.5 × 105 Pa vacuum degrees.

To analyze the effect of CNTs on the bioactivity, the scaffolds specimens were immersed in simulated body fluid (SBF) following the method developed by Kokubo et al.31) and maintained at 37°C for 10 days. Each sample was soaked in 20 ml of SBF with the constant solid mass/liquid volume ratio (0.1 g/100 ml), and the SBF solution was replaced every 1 day to simulate the recirculation of physiological fluid and to contribute to the HCA formation.32) After being soaked for 10 days, the scaffolds specimens were rinsed with the deionized water and acetone, and then dried in air at room temperature. The formation of the HCA layer on the surfaces of the scaffolds specimens was confirmed by SEM (as described above) and FTIR analysis. FTIR analysis was operated on the Fourier Transform Infrared Spectroscopy (NicoletoTM 6700 spectrometer, Thermo Scientific Co. USA) in mid-infrared (MIR) region of 400–4000 cm⁻¹. For FTIR analysis, the powder of the tested samples of 1 mg were mixed with 300 mg of KBr (infrared grade) and were pressed into a pellet under vacuum. Then, the pellet was analyzed at the scan speed of 0.6329 cm/s with 4 cm⁻¹ resolution.

Human osteosarcoma MG-63 cells were used to evaluate the cell adhesion of the scaffolds specimens. Before seeding cells, the scaffolds specimens were sterilized with 70% ethanol for 30 min and then further sterilized under UV light for 30 min, followed by drying under sterile conditions at room temperature for 2 h. Then, the cells were directly seeded on the scaffolds specimens for 6 days. After culture, the scaffolds specimens with the attached cells were washed with phosphate-buffered saline (PBS) and dehydrated in graded ethanol (30–100%) and freon. Finally, the morphologies of the cells on the surface of the scaffolds specimens were analyzed by SEM.

3. Results and discussion

The fracture toughness and Vickershardness of the nanocomposite scaffolds with the different amounts of CNTs were analyzed. [Fig. 1(a)]. The fracture toughness initially increased
with increasing weight fraction of CNTs from 1 to 3 wt.%. The maximum value of the fracture toughness was 1.58 MPa·m$^{1/2}$. This was due to the strong interfacial bonding of 13–93 bioactive glass and CNTs and the uniform dispersion of CNTs in the 13–93 bioactive glass matrix (discussed below). However, the fracture toughness reduced with the further increase in the CNTs weight fraction to 4 or 5 wt.%. The decrease was because that the agglomeration of CNTs weakened the bonding between the CNTs and the 13–93 bioactive glass and decreased the nanocomposite scaffolds relative density.

The effects of CNTs on the Vickers hardness of the nanocomposite scaffolds were analyzed. Vickers hardness gradually decreased from 583.9 to 506.7 HV with increase in the amount of CNTs. Hardness was a measure of the strength of the material and this slightly decrease was partly attributed to the addition of the relatively soft CNTs into the hard 13–93 bioactive glass matrix. The result was also in agreement with the hardness data of CNT composites published by Amartya and Tjong.\textsuperscript{33,34}

The compressive strength and Young’s modulus of the scaffolds with the different amounts of CNTs were discussed [Fig. 1(b)]. The compressive strength and Young’s modulus showed a similar trend as the fracture toughness. After addition of 3 wt.% CNTs, the compressive strength increased markedly from 21.35 to 37.32 MPa due to the CNTs dispersion in the matrix as a reinforcing

![Fracture toughness and Vickers hardness](image1.jpg)

**Fig. 1.** Variation of: (a) fracture toughness and Vickers hardness, (b) compressive strength and Young’s Modulus of the 13–93 glass-CNTs nanocomposite scaffold as a function of CNTs amount.

![Surface morphologies](image2.jpg)

**Fig. 2.** Surface morphologies of 13–93 glass, CNTs, and the nanocomposite power and scaffold. (a) the original 13–93 glass, (b) the original CNTs, (c) the 13–93 glass-1 wt.% CNTs nanocomposite powder, (d) the 13–93 glass-1 wt.% CNTs nanocomposite scaffold after sintering.
phase. Accordingly, Young’s modulus increased from 120.3 to 149.1 MPa. Further adding CNTs to 5 wt.%, the compressive strength and Young’s modulus reduced to 28.43 and 127.2 MPa, respectively, due to the agglomeration of CNTs.

The surface morphologies of 13–93 glass and CNTs before and after mixing were analyzed (Fig. 2). The original 13–93 glass had short rod-shape nanostructure [Fig. 2(a)]. The original CNTs had the fiber structure, and the average diameter was about 30 nm [Fig. 2(b)]. For 13–93 glass-1 wt.% CNTs nanocomposite powder, the CNTs were uniformly distributed in the 13–93 glass [Fig. 2(c)]. And after sintering, the CNTs were also homogeneously dispersed [Fig. 2(d)].

The surface morphologies of the nanocomposite scaffold with 3 and 4 wt.% CNTs were presented in Fig. 3. When the amount of CNTs was 3 wt.%, the CNTs are homogeneously dispersed in the 13–93 bioactive glass [Fig. 3(a)]. However, further increasing the amount to 4 wt.%, the dispersion of CNTs was not very uniform due to their agglomeration [Fig. 3(b)]. So, the optimum content of the CNTs was 3 wt.%, which made them to uniformly disperse in the 13–93 bioactive glass. And above this value,
CNTs generated agglomeration and impaired the mechanical properties of the nanocomposite scaffolds.

A representative crack propagation path was obtained on the 13–93 glass-3 wt.% CNTs nanocomposite scaffold using micro-hardness indentation at a maximum load of 4.9 N [Fig. 4(a)]. The high-resolution image of the crack propagation paths was shown in Fig. 4(b). It was observed that there were crack bridging, pull-out and crack deflection induced by the CNTs on the crack surface. In order to investigate the toughening mechanisms induced by CNTs, the schematic diagram of crack bridging, pull-out and crack deflection [16,19,33,34] were shown in Figs. 4(c)–4(e). The crack area existed at the 13–93 glass-CNTs interface of the tip of the crack, and was bridged by the CNTs [Fig. 4(c)]. In the crack area, CNTs exerted closure stress on the crack surface and reduced the subjected external forces of the crack tip. And the crack bridging by CNTs restrained crack propagation and played a toughening effect. Thus, the bridging effect of CNTs improved the fracture toughness of the nanocomposite scaffolds.

Besides the crack bridging, pull-out was another type of the toughening mechanism. When the crack encountered the high intensity CNTs, the large shear stresses easily made the CNTs to pull out from the matrix. That was to say, CNTs was pull-out from one side of the fracture surface and a hole was left in the opposing face [Fig. 4(d)]. In this process, CNTs pull-out made the crack tip stress relaxation and needed to provide the external force to do work. So, CNTs pull-out had favorable toughening effect in the 13–93 glass-CNTs nanocomposite scaffolds. The third toughening mechanism was crack deflection. When the main crack reached to CNTs, it must bypass the CNTs to continue to propagate due to the super-strong mechanical properties of the CNTs. It meant that the propagation direction of the crack was altered and the crack deflection occurred [Fig. 4(e)]. In this process, the crack propagation path and new crack surface area increased. Accordingly, the stress intensity at the crack tip was reduced. So, the fracture toughness of the 13–93 glass-CNTs nanocomposite scaffolds increased by virtue of crack deflection. In conclusion, the incorporation of the CNTs toughed the 13–93 glass-CNTs nanocomposite scaffolds by crack bridging, pull-out and crack deflection.

In order to investigate the bioactivity of the 13–93 glass-CNTs nanocomposite scaffolds, The formation of an HCA layer on the surface of the monolithic 13–93 glass scaffold and the 13–93 glass-3 wt.% CNTs nanocomposite scaffold by incubating in simulated body fluid (SBF) for 10 days was confirmed [Figs. 5(a) and 5(b)]. For the monolithic 13–93 glass scaffold [Fig. 5(a)], the layer of HCA crystals with globular shape covered on the surface of the scaffold due to the ionic exchange between the scaffold’s surface and the solution. The homogenously layer of HCA crystals with well-known slender needles shape covered on the surface of the 13–93 glass-3 wt.% CNTs nanocomposite scaffold [Fig. 5(b)]. What’s more, HCA crystals existed in the form of the nano-sized structure. To determine the components of HCA on the surface of the nanocomposite scaffold, the FTIR spectra of the scaffold before and after soaking were analyzed [Figs. 5(c) and 5(d)]. For the FTIR spectra of the scaffold before soaking [Fig. 5(c)], the absorption band at 1038 cm⁻¹ corresponded to unsymmetry flex vibration (ν3) of PO4³⁻, 470 cm⁻¹ corresponded to Si–O–Si bending vibration.
mode. After soaking [Fig. 5(d)], the silicate absorption intensity decreased, a couple of well-defined broadband appeared at 606 and 573 cm⁻¹ corresponded to P-O bending vibrations, and some CO₂⁻ groups was observed around 1400–1600 cm⁻¹, which further demonstrated the existence of HCA crystals on the scaffolds. The results confirmed that the 13–93 glass-3 wt.% CNTs nanocomposite scaffold had bioactivity. Moreover, the addition of CNTs can induce the formation of an ordered nanostructured HCA layer. This was because that bioactive glass was a well known bioactive material and the HCA layer formed on its surface by direct activation of Bioglass dissolution products. CNTs with the negatively charge promoted mineralization by their enhanced ability to nucleate HCA on their surfaces, as found by other author.39 In particular, the presence of CO₂⁻ groups had been proved to induce the precipitation of nanocrystals of HCA from SBF in biomimetic methods.37,38 In order to evaluate the cytocompatibility of the 13–93 glass-CNTs nanocomposite scaffolds, an osteoblast proliferation study using MG-63 bone-forming cells after 10 days was cultured on the monolithic 13–93 glass and the 13–93 glass-3 wt.% CNTs nanocomposite scaffolds (Fig. 6). It was seen that many MG-63 cells were covered on the whole surfaces of the both scaffolds, MG-63 cells had the ellipsoidal structure. Therefore, the 13–93 glass-3 wt.% CNTs nanocomposite scaffold could sustain MG-63 cells growth and proliferation, which indicating that the 13–93 glass-CNTs nanocomposite scaffolds had good cytocompatible. This was because that CNTs carried the neutral electric charge, and were favourable for cell growth, which was consistent with the results obtained by others.39,40 The 13–93 glass-3 wt.% CNTs nanocomposite scaffold was fabricated by selective laser sintering according to the abovementioned processing parameters (Fig. 7). The scaffold was the trapezoidal shape with 12.5 mm in height, 12 and 7.5 mm in length. And after the non-sintered composite powders entrapped in the pores of the scaffold was removed by a compressed air jet, the scaffold had the complete interconnected porous structure. The strut and pore sizes were approximately 1 and 1.2 mm in width, respectively. The porosity of the scaffold was about 42.01%. In the meantime, the results also demonstrated that SLS had the capability to fabricate porous scaffolds in accordance with computer-aided design.

4. Conclusions

The 13–93 glass-CNTs nanocomposite scaffolds had been fabricated by selective laser sintering. The effects of CNTs on the mechanical and biological behavior of 13–93 glass and the toughening mechanism were investigated. The results showed that when the optimal amount of CNTs was 3 wt.%, the compressive strength and fracture toughness of nanocomposite scaffolds increased by 75% (up to 37.32 MPa) and 49% (up to 1.58 MPa·m²/2), respectively. The increase was attributed to the crack bridging, pull-out and crack deflection induced by CNTs. The Vickershardness gradually decreased with an increase in the concentration of CNTs. This was because of agglomeration and relatively low density of CNTs. In addition, the results of SBF test and MG-63 cells culture indicated that the nanocomposite scaffolds had the good bioactivity and facilitated cells growth and proliferation. These results suggested that 13–93 glass-CNTs nanocomposite scaffolds, up 3% CNTs has not only the improved fracture toughness and compressive strength, but also the good bioactivity and cell biocompatibility, demonstrating a promising development in bone tissue engineering scaffolds.

Acknowledgements This work was supported by the following funds: (1) The Natural Science Foundation of China (51405521, 51222506, 81372366); (2) Overseas, Hong Kong & Macao Scholars Collaborated Researching Fund of National Natural Science Foundation of China (81428018); (3) Hunan Provincial Natural Science Foundation of China (12JJ4067,14JJ1006); (4) Project supported by the Fork Ying-Tong Education Foundation, China (131050); (5) Shenzhen Strategic Emerging Industrial Development Funds (JCYJ20130401160614372); (6) The Open-End Fund for the...
Valuable and Precision Instruments of Central South University; (7) The faculty research grant of Central South University (2013JSJJ011, 2013JSJJ046); (8) State Key Laboratory of New Ceramic and Fine Processing Tsinghua University (KF201411).

References