Research Article

Crystallinity and Reinforcement in Poly-L-Lactic Acid Scaffold Induced by Carbon Nanotubes

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Poly-L-Lactic Acid (PLLA) is a bioabsorbable implant material due to its favorable biocompatibility and inherent degradability, while the insufficient mechanical strength hinders its further bone repair application. In present work, carbon nanotubes (CNTs) were introduced into PLLA scaffolds fabricated via selective laser sintering. It was found that the crystallinity of PLLA increased considerably since CNTs could promote the orderly stacking of its molecular chains, thereby improving the mechanical strength of PLLA scaffold. Furthermore, the fracture surface analysis revealed that CNTs acted as a bridge across the cracks and hindered their further expansion. Moreover, CNTs pulled out from the matrix to consume a large amount of fracture energy, which enhanced the resistance to external forces. As a consequence, the compressive strength, Vickers hardness and tensile strength of the scaffold were enhanced by 22.7%, 58.8% and 17.6%, respectively. Besides, the cells exhibited good attachment, spreading and proliferation on the scaffold. This study demonstrated that PLLA/CNTs scaffold was a promising candidate as bone implant.

1. Introduction

Poly-L-Lactic Acid (PLLA) has been recognized as a promising bone repair material due to its good biocompatibility and desirable biodegradability [1–3]. It is able to degrade into lactic acid by hydrolysis, and then metabolize to water and carbon dioxide, which can be removed from the body via normal metabolic pathways [4, 5]. Given the attractive characteristic of PLLA, it has received the approval from the Food and Drug Administration for implant application [6]. Recently, numerous investigations have attempted to utilize PLLA as temporary bone implant [7, 8]. Nevertheless, these studies indicated that PLLA exhibited poor mechanical properties, especially for load bearing, which significantly hinder its further bone repair application [9].

Incorporation of reinforcement, such as nanoparticles [10, 11], nanowires [12], graphene nanoplatelets [13] and carbon nanotubes (CNTs) [14], is a workable approach to enhance the mechanical performance of polymer matrix. Among them, CNTs exhibited superior mechanical performance, including high strength, modules and hardness [15]. Meanwhile, CNTs have been proved to be of favorable biocompatibility and osteo-protective property [16]. Li et al. introduced CNTs into polycarbonate microfibrils, and enhanced the tensile strength and elastic modulus by 20%, 40%, respectively [17]. Wang et al. proved that CNTs as a nanofiller for PLLA could overcome the brittleness, with the elongation at break and the impact strength improved 205% and 30%, respectively [18]. Mesgar et al. used CNTs to reinforce the chitosan scaffolds, which enhanced the modulus to 11 times [19]. Luo et al. fabricated multi-walled carbon nanotubes polycaprolactone composite scaffolds using the solution evaporation technique [20]. In vitro results showed that the composite scaffolds had good bone bioactivity.

In present study, CNTs were incorporated into PLLA to enhance the mechanical properties. The PLLA/CNTs composite scaffold was prepared by selective laser sintering (SLS) process. Previous researchers have fabricated PLLA/CNTs scaffold via phase separation method [21], electrospinning technique [22], freeze-drying method [23], etc. Comparing
with previous methods, selective laser sintering exhibits great advantages including accurate control of the pore structure and strong ability to customize for specific defect sites [24–27]. The microstructure evolution and mechanical behavior of the composite scaffold were investigated. The effect of CNTs on the mechanical behavior was clarified in detail. In addition, the biocompatibility of the PLLA/CNTs scaffold was studied via in vitro cell experiments.

2. Materials and Methods

2.1. Materials. CNTs with the outside diameter ranging from 4 to 6 nm and the length ranging from 10 to 20 µm was provided from Chengdu Organic Chemicals Co. Ltd. The PLLA powder was purchased from Shenzhen Polymtek Biomaterial Co. Ltd. (Shenzhen, China) with average molecular weights 150 mol/g, purity more than 99%, and density 1.25 g/cm³.

2.2. Preparation of PLLA/CNTs Composite Scaffold. The detailed fabrication process of PLLA/CNTs scaffold was depicted in Figure 1. Specifically, a certain quantity of CNTs was first added into anhydrous ethanol solution. The suspension was then magnetically stirred and ultrasonically dispersed for 6 h. Subsequently, the PLLA powder was added into the above CNTs suspension. Afterwards, the PLLA/CNTs suspension was magnetically stirred and ultrasonically dispersed for 12 h. The suspension was then dried in a vacuum oven at 45°C until the powder weight maintained stable. Finally, the PLLA/CNTs mixed powder was obtained after ball milling for 1 h.

The PLLA/CNTs composite scaffold was prepared using a self-developed SLS system. The detailed preparation process was described as follows: Firstly, the 3D model matching the bone scaffold was designed. The model was imported into the SLS system to create the slice file; secondly, the composite powder was spread on the workbench; thirdly, the composite powder was selectively sintered layer-by-layer according to the above slice file. The optimal process parameters of SLS process were determined by pilot experiments (laser power 2.5 W, scanning speed 120 mm/s, scanning distance 0.1 mm and layer thickness 0.1 mm). The obtained scaffolds with nominal CNTs of 0, 0.25, 0.5, and 1.0 wt.% were named as PLLA, PLLA/0.25CNTs, PLLA/0.5CNTs, and PLLA/1.0CNTs, respectively. The pore size and strut size of the sintered scaffolds were about 650 µm and 450 µm, respectively, as shown in Figure 1. In general, porous bone scaffolds was able to provide a favorable microenvironment for cell into-growth [28, 29].

2.3. Microstructural Characterization. The chemical characteristic of the samples was qualitatively evaluated by Fourier transform infrared spectroscopy (FTIR, Bruker Tensor 27). The samples and dried potassium bromide powder were mixed and grounded into fine powder. Then, they were pressed into discs in a mold. The FTIR spectra were obtained with a resolution of 2 cm⁻¹ from 500 to 3000 cm⁻¹ at room temperature. Subsequently, the intensity of transmittance and the characteristic peaks was measured. Raman spectroscopy was obtained using a Raman spectrometer (Renshaw, UK) with a laser excitation of 532 nm and scans range between 400 and 2500 cm⁻¹. Polarized optical microscope (POM, Olympus Co., Japan) was used to investigate the crystalline morphology of PLLA/CNTs composites. The phase structure was investigated using an X-ray diffractometer (XRD, DMAX 2500, Japan Science Co., Japan) equipped with Cu Ka source. The data was recorded ranging from 10° to 50°.
2.4. Mechanical Tests. The Vickers hardness tester (TMVS-1, Beijing TIME High Technology Co. Ltd) was applied to measure the hardness. Three different measurements were carried out for each sample. A mechanical testing machine (WD-D1, LTD, China) was applied to perform the tensile and compressive tests. The tensile specimens (width 5 mm, length 20 mm) was subjected to tensile load until they were broken, and the compressive specimens with porous structure (10 × 10 × 5 mm³) were subjected to compression load until they were crushed. The fracture surface morphologies of the scaffolds after tensile tests were observed using a scanning electron microscope (Phillips XL30, Eindhoven, Netherlands). Before SEM observation, the surface of samples was coated with gold for 2 min in a sputter coater.

2.5. Cell Experiments. MG63 cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% (v/v) streptomycin/penicillin condition at 37°C (5% humid CO₂). The culture medium was changed every 2 days. Prior to cell seeding, the PLLA and PLLA/CNTs scaffold (10 × 10 × 2 mm³) were sterilized under UV irradiation for 24 h. The incubate medium was renewed every day. The MG63 cells were seeded on the PLLA and PLLA/CNTs scaffolds with a density of 10⁵ cells per well. After 1, 4 and 7 days, the specimens were rinsed three times using phosphate buffer solution (PBS) in order to remove nonadherent cells. The cell morphology on scaffolds with different culture time was observed by SEM.

The cell viability was assessed using live/dead staining after culture for 1, 4, 7 days. In brief, the cells were stained with

FiguRe 2: (a) FTIR and (b) Raman spectra of CNTs, neat PLLA, and PLLA/CNTs scaffolds.

FiguRe 3: SEM showing the fracture surface of (a) PLLA, (b) PLLA/0.25CNTs, (c) PLLA/0.5CNTs, and (d) PLLA/1.0CNTs scaffolds.
2.6. Statistical Analysis. All the experimental data were recorded using at least three separate operations, and expressed as means ± standard deviation. In all statistical analysis, it is considered to be significant with $P$ value less than 0.05.

3. Results and Discussion

3.1. Characterization of the PLLA/CNTs Composite Scaffolds. The FTIR spectra of CNTs, PLLA and PLLA/CNTs scaffolds were displayed in Figure 2(a). As was expected, there was a peak presented at 1635 cm$^{-1}$, which was assigned to the stretching vibrations of C=C bonds in the CNTs. For PLLA, there were two obvious peaks at 1751 and 1452 cm$^{-1}$, which was ascribed to the C=O stretching vibrations of its carboxyl groups [30]. The peak located at 1380 cm$^{-1}$ was assigned to the bending vibrations of $\sim$CH$_3$ groups [31]. There was a weak broad band appearing at 1183 cm$^{-1}$ which was associated to C-O stretching vibration [32]. Compared with PLLA, there were no new peaks or peak shift in the FTIR spectra of PLLA/CNTs scaffolds.

The Raman measurements were used to further reveal the structural stability of the CNTs during the SLS process. The Raman spectra of CNTs, PLLA and PLLA/CNTs composite scaffolds were recorded, with results shown in Figure 2(b). It could be found that there were two distinct characteristic peaks observed at 1330.6 and 1656.7 cm$^{-1}$, which was ascribed to the D- and G-bands of CNTs, respectively. Generally, the D-band was related to the degree of disorder of CNTs, while the G-band was attributed to the in-plane motion of the sp$^2$ carbon atom.
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The number of micro-fibers increased with the CNTs contents increasing to 0.5 wt.%. However, the agglomeration occurred with CNTs further increasing to 1.0 wt.%.

The crystalline morphology of PLLA/CNTs was investigated using POM, with results displayed in Figure 4. Clearly, PLLA exhibited large spherulites morphology with a typical maltese cross shape. As CNTs gradually increased to 0.5 wt.%, the size of the spherulites decreased considerably, accompanying with the significantly increased density. The results indicated that CNTs acted as heterogeneous nucleating agents and promoted the orderly arrangement, thus accelerating the crystallization of PLLA. Nevertheless, as the CNTs reached 1.0 wt.%, relatively coarsened spherulites were observed in the matrix, indicating the reduced nucleation ability of CNTs for composite matrix. Previous studies revealed that as nanoparticles exceeded a certain content, the ability to promote PLLA nucleation would decrease, which was believed to be related to their agglomeration in PLLA matrix [35].

[4, 33]. PLLA exhibited three characteristic peaks at 859.6, 1083.8 and 1444.8 cm⁻¹, which were attributed to C–CH₃ stretching, −CH₃ rocking mode and −CH₃ asymmetric bending mode, respectively [34]. For the Raman spectrum of PLLA/CNTs scaffolds, they presented combined characteristic peaks of PLLA and CNTs. The D and G modes of the scaffolds exhibited no noticeable shifts as compared with the spectrum of CNTs. However, the PLLA resonances in the scaffolds included C–CH₃ stretching (861.2 cm⁻¹), −CH₃ rocking mode (1085.3 cm⁻¹), and −CH₃ asymmetric bending mode (1446.2 cm⁻¹) were red-shifted by 1.6, 1.5 and 1.4 cm⁻¹, respectively, as compared with the pure PLLA scaffold [4]. It might be due to the interaction between PLLA and CNTs during SLS.

The brittle fractured surfaces of PLLA/CNTs scaffolds were observed, as shown in Figure 3. PLLA exhibited an obvious brittle fracture with relative flat morphology. As a comparison, there were some micro-fibers on the fractured surface of the PLLA/0.5CNTs scaffold. The CNTs uniformly distributed in the PLLA matrix. The number of micro-fibers increased with the CNTs contents increasing to 0.5 wt.%. However, the agglomeration occurred with CNTs further increasing to 1.0 wt.%.

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Figure 6: Tensile stress-strain curves (a), tensile strength (b), compressive strength (c), and hardness of PLLA and PLLA/CNTs composite scaffolds (d).
The XRD patterns of PLLA and PLLA/CNTs were shown in Figure 5. Obviously, there were two strong diffraction peaks located at $2\theta = 16.8^\circ$ and $19.1^\circ$ [36], which indicated the occurrence of cold crystallization of PLLA [37]. The XRD patterns of the PLLA/CNTs scaffold still kept the characteristic peaks of PLLA, which indicated that the incorporation of CNTs did not exert a significant influence on the crystalline structure of PLLA. However, the intensity and sharpness of these peaks gradually increased with CNTs increasing, which indicated that the degree of crystallization of polymer composite was enhanced due to the heterogeneous nucleation of CNTs [30].

3.2. Mechanical Properties. Bone scaffolds require appropriate mechanical strength to provide structural support for
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The mechanical properties of PLLA and PLLA/CNTs scaffolds were evaluated through compressive, hardness and tensile tests, with results shown in Figure 6. The typical compressive stress-strain curves were depicted in Figure 6(a). It could be found that all the samples exhibited the brittle characteristic. As the external force exceeded a certain degree during the compression, the scaffolds were directly fractured without yielding [40]. The compressive strengths derived from their compressive stress-strain curves were shown in Figure 6(b). The compressive strengths of PLLA/CNTs scaffolds increased by 22.7% (from 5.5 MPa to 6.82 MPa) with CNTs increasing to 0.50 wt.%. This could be ascribed to the fact that CNTs could act as a reinforced phase in the PLLA matrix [41]. However, there was a slight decrease in compressive strength with CNTs further increasing to 1.0 wt.%, which might be due to the aggregation of CNTs. It was well established that bone scaffolds should have sufficient mechanical strength to match the newly formed tissue during bone repair [38, 39].

![Representative fluorescence images of MG63 cells cultured on scaffolds for 1, 4 and 7 days.](image)

**Figure 9:** Representative fluorescence images of MG63 cells cultured on scaffolds for 1, 4 and 7 days.

![The obtained OD value using CCK-8 assays after 1, 4, 7 days’ culture.](image)

**Figure 10:** The obtained OD value using CCK-8 assays after 1, 4, 7 days’ culture.
the surrounding connective bone. Usually, the compressive strength of the human cancellous bone and cortical bone were in the range of 2–12 MPa and 130–180 MPa, respectively [42]. In this work, the optimal compressive strength of PLLA/CNTs scaffolds was 6.8 MPa, which was in the range of cancellous bone and lower than cortical bone. Therefore, the PLLA/CNTs scaffolds have a potential application in bone repair.

In this work, the CNTs could act as a pinning action to hinder the crack propagation. This was due to the crack in the composite was difficult to deflect during the expansion process and could only continue in the original direction [43]. Thus, the CNTs immediately adjacent to the crack tip did not break, but created a bridging effect between the cracks [44, 45]. The typical crack bridge in the crack surface contact region was shown in Figure 7(a). This bridging effect would offset a part of the tensile stress, thereby hindering the further expansion of the crack. On the other hand, CNTs, as one dimensional material, exhibited significant fiber properties [46]. In this case, the CNTs were pulled out of the matrix during the tensile fracture of the composite scaffolds, thereby consuming a large amount of external energy [47]. As shown in Figure 7(b), some CNTs pulled out from the matrix, whose direction tended to align with the direction of the applied load. Besides, CNTs were embedded into PLLA matrix, which formed the pinning effects. The schematics of pulling out, bridging and crack deflection are shown in Figure 7(d). Furthermore, the increase in the crystallinity of PLLA also had a positive effect on their mechanical properties [48]. However, with the CNTs contents further increasing to 1.0 wt.%, they aggregated in the PLLA matrix, thus failing to enhance the mechanical properties of PLLA/CNTs scaffolds.

3.3. Cytocompatibility. Good biocompatibility was of great significance for the bone implants [49–51]. In present study, the PLLA/0.5CNTs scaffold with optimal mechanical properties was used to further evaluate the cytocompatibility, with the PLLA scaffold as control. The morphologies of cells adhered on scaffolds after cultivating for 1, 4, 7 days were observed by SEM, as shown in Figure 8. After 1 day culture, cells began to spread on the PLLA/0.5CNTs scaffold with apparent cytoplasmic extensions, indicating a strong adhesion. As culture time increasing to 4, 7 days, the adhered cells on the PLLA/0.5CNTs scaffold increased significantly and overlapped with each other. Additionally, the cells on the PLLA/0.5CNTs scaffold presented much longer filopodia as compared with those on PLLA scaffold. It was indicated that PLLA/0.5CNTs scaffold was more beneficial for cell adhesion.

Stained fluorescent pictures of cells incubated on PLLA/0.5CNTs and PLLA scaffolds for various periods were exhibited in Figure 9. Herein, green represented live cells, whereas red represented dead cells. Clearly, nearly no dead cells were observed for all the samples during cell incubation. In particular, the cells exhibited typical fusiform shape after culture for 1 day, revealing that the cells growth normally on the scaffold. After further incubation for 4, 7 days, cells have expanded into filopodia, and formed numerous extracellular matrix. At the same time, live cells increased significantly with incubation time increasing, which indicated that both PLLA/0.5CNTs and PLLA scaffolds were favorable for cell proliferation. It should be noted that the PLLA/0.5CNTs scaffold exhibited a higher cell density than PLLA scaffold, indicating that it was more beneficial to cell growth.

The viability of cells incubated on PLLA/0.5CNTs and PLLA scaffolds was quantitatively assessed by CCK-8 assay, with results shown in Figure 10. It could be seen that the cell viability on PLLA/0.5CNTs and PLLA scaffolds increased with culture period extending from 1 to 7 days. In detail, the OD values increased from 0.6 to 2.8 for PLLA/0.5CNTs scaffold. Significantly, PLLA/0.5CNTs scaffolds presented enhanced OD values comparing with the PLLA scaffold at same time, which further proved that CNTs shown a positive effect on cell proliferation.

Basing on above cell experiments, it was indicated that PLLA/CNTs scaffold exhibited an enhanced cytocompatibility for cell adhesion and growth, as compared with PLLA scaffold. In general, cells were able to distinguish surfaces with different physical, chemistry and mechanical properties that directly affected cell behavior. As cells adhered to scaffold surface, they created a larger force at the adhesion site. It was believed that the PLLA/CNTs scaffold with stiffer matrix than PLLA scaffold could provide better cell adhesion. Secondly, the incorporation of CNTs into PLLA matrix resulted in the rough nano-scaled surface topography, which was more conducive for cell adhesion and growth [36]. In fact, rough surface was able to provide a variety of mechanical stimuli, thus promoting cell proliferation. Besides, the incorporated CNTs with excellent electrical properties could enhance the transmission of cell signals, thus promoting cell–cell communication and cellular activity [52].

4. Conclusions

In present study, CNTs were introduced into PLLA to improve the mechanical behavior. PLLA/CNTs scaffold was fabricated by SLS technique. CNTs enhanced the crystallinity of PLLA matrix due to its heterogeneous nucleation effect. More significantly, CNTs, as one-dimensional material, contributed to the crack bridging mechanism and pulling out effect. As a consequence, the obtained compressive strength, Vickers hardness and tensile strength of the scaffold were considerably enhanced from 5.6 to 6.8 MPa, 24.5 to 38.9 Hv, 15.5 to 18.6 MPa, respectively. Furthermore, PLLA/CNTs scaffold exhibited desirable biocompatibility. It was indicated that PLLA/CNTs scaffold was a promising candidate as bone implant.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.
Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors’ Contributions
Guoyong Wang and Fangwei Qi contributed equally in this work.

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