Fabrication and in vitro biocompatibility of biomorphic PLGA/nHA composite scaffolds for bone tissue engineering

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In this study, biomorphic poly(\(\epsilon\)-lactic-co-glycolic acid)/nano-hydroxyapatite (PLGA/nHA) composite scaffolds were successfully prepared using cane as a template. The porous morphology, phase, compression characteristics and in vitro biocompatibility of the PLGA/nHA composite scaffolds and biomorphic PLGA scaffolds as control were investigated. The results showed that the biomorphic scaffolds preserved the original honeycomb-like architecture of cane and exhibited a bimodal porous structure. The average channel diameter and micropore size of the PLGA/nHA composite scaffolds were 164 ± 52 μm and 13 ± 8 μm, respectively, with a porosity of 89.3 ± 1.4%. The incorporation of nHA into PLGA decreased the degree of crystallinity of PLGA, and significantly improved the compressive modulus of biomorphic scaffolds. The in vitro biocompatibility evaluation with MC3T3-E1 cells demonstrated that the biomorphic PLGA/nHA composite scaffolds could better support cell attachment, proliferation and differentiation than the biomorphic PLGA scaffolds. The localization depth of MC3T3-E1 cells in the channels of the biomorphic PLGA/nHA composite scaffolds reached approximately 400 μm. The results suggested that the biomorphic PLGA/nHA composite scaffolds were promising candidates for bone tissue engineering.

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1. Introduction

Bone tissue engineering has been considered to hold great promise for bone regeneration, and requires a biomimetic three-dimensional scaffold mimicking the porous structure and extracellular matrix (ECM) functions of the bone. Various porous scaffolds with different structures have been successfully prepared by a variety of techniques. Among currently available methods, preparing biomorphic scaffolds from natural biological tissues is considered a very good choice, which is based on the postulate that only tissue/organ-derived ECMs possess the true histostucture and are theoretically best able to facilitate tissue regeneration [1,2]. Therefore, there is a large demand for biomorphic scaffolds in the field of tissue engineering. Classical biomorphic scaffolds include demineralized bone matrices, calcined animal bones and decellularized ECMs derived from various tissues [3,4]. These tissue-derived scaffolds are very attractive in bone tissue engineering, because they can not only provide native intricate three-dimensional architectures of original tissues but also retain the biological and physico-chemical properties of native insoluble ECM components [5]. The naturally derived scaffolds are being intensively studied for tissue engineering such as the osteochondral tissue [6], cartilage [7], bone [3,8], and smooth muscle [9]. However, these scaffolds have some unavoidable issues, such as limited allogeneic donor tissues, uncontrollable degradation rate, poor strength, immunogenicity, and potential risk of infection [10,11]. Therefore, there is still a strong and urgent need to develop novel biomorphic scaffolds to mimic the unique hierarchical architecture of the bone.

The researchers in the fields of materials and biomedical sciences have been attempting to explore new biomimetic technologies to fabricate biomorphic scaffolds to mimic the porous structure of native bone. For example, the skeletons of some ocean animals such as cuttlefish [12], coral [13], sea star [14], and mollusk shell [15], which are made of calcium carbonate, could be transformed into hydroxyapatite or biphasic calcium phosphate by chemical reactions [16]. The naturally derived bioceramics could be used for bone regeneration [16], but their pore sizes are not in the optimal pore size range (100–400 μm) for bone tissue engineering scaffolds [14,17,18]. In the past years, novel biomorphic hydroxyapatite scaffolds have been successfully prepared using wood as a template through different transformation routes [19,20]. It is well known that wood possesses a specific hierarchical porous structure pseudomorphous to sponge bone. Several early observations on bone repair with wood implants have verified that the unique pore structure of wood could promote host bone growth into wood implants and allowed cartilage differentiation on their surface [21,22]. Therefore, wood-derived biomorphic biomaterials would possess a combination of the unique cellular structure of wood and the excellent biological properties of biomaterials, representing a promising approach to obtain biomimetic scaffolds for tissue engineering. However, the porous structure of wood could only be transformed into inorganic biomaterials through current techniques. Recently, we presented a novel process by which biomorphic biopolymer-based scaffolds could...
be fabricated using cane as a template [23]. This endowed the flexible material design of biomorphic scaffolds to better satisfy the requirements of tissue engineering.

Both naturally derived and artificially synthesized biopolymers have been widely used as scaffolding materials in the field of tissue engineering [24,25]. As one of the most successfully developed biopolymers [26], poly(3-lactic-co-glycolic acid) (PLGA) possesses good biodegradability, bioabsorbability, biocompatibility and processability, and it has been extensively studied in a variety of tissue engineering applications, such as the skeletal muscle [27], bone [28], cartilage [29], ligament/ten
don [30], and nerve [31]. However, in bone tissue engineering, the application of PLGA is greatly limited by low mechanical strength, hydrophobic surface and poor bioactivity [32]. Some studies have demonstrated that the incorporation of inorganic nanoparticles including hydroxyapatite and carbon nanotube into PLGA could efficiently solve the above-mentioned problems [33–35]. Hence, the PLGA-based nano
composite materials are promising for bone tissue engineering applications.

In the present study, biomorphic PLGA/nHA composite scaffolds were fabricated using cane as a template via the transformation process developed by us. The morphology, physicochemical properties and in vitro biocompatibility of the PLGA/nHA composite scaffolds were investigated, using biomorphic PLGA scaffolds as control. It was found that both scaffolds well preserved the porous morphology of cane at micrometer scale, and that the biomorphic PLGA/nHA composite scaffolds could better support the attachment, spreading, proliferation and osteogenic differentiation of MC3T3-E1 cells, compared to the biomorphic PLGA scaffolds. The preliminary results confirmed the potential of biomorphic PLGA/nHA composite scaffolds in bone tissue engineering.

2. Experimental procedure

2.1. Materials

Poly(3-lactic-co-glycolic acid) (75:25 PLGA, Mn: ~2 × 105 g/mol) was supplied by Daigang Biomaterials Co. (Shandong, China). Hydroxyapatite nanospheres (nHA, less than 100 nm in size) were purchased from Aladdin Reagent Co. (Shanghai, China). Pre-osteoblast-like MC3T3-E1 cells were obtained from the Medical Center of Xi’an Jiaotong University (Xi’an, China). Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Gibco. Betaine bovine serum (FBS) was obtained from Hangzhou Sijiqing Biological Technology Co., Ltd. (Zhejiang, China). Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Gibco. Betaine bovine serum (FBS) was obtained from Hangzhou Sijiqing Biological Technology Co., Ltd. (Zhejiang, China). Poly(3-lactic-co-glycolic acid) (PLGA) possesses good biodegradability, bioabsorbability, biocompatibility and processability, and it has been extensively studied in a variety of tissue engineering applications, such as the skeletal muscle [27], bone [28], cartilage [29], ligament/ten
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2.2. Preparation of biomorphic scaffolds

Biomorphic PLGA and PLGA/nHA composite scaffolds were prepared as described in our previously reported procedure [23]. Briefly, the negative NaCl templates of cane were firstly prepared by calcining dense cane/NaCl complex samples at 780 °C for 2 h in air. Secondly, the porous NaCl templates were filled with PLGA solution or PLGA/nHA composite suspension in dichloromethane under vacuum, and then dried. The impregnation process was repeated three times. Finally, NaCl in the PLGA/NaCl or PLGA/nHA/NaCl complex samples was removed by soaking in distilled water, resulting in biomorphic PLGA or PLGA/nHA composite scaffolds. In the experiment of preparing PLGA/nHA composite scaffolds, the weight ratio of nHA to PLGA was 1:19.

2.3. Scaffold characterization

The morphology and porous structure of cane template and biomorphic PLGA and PLGA/nHA composite scaffolds were observed by scanning electron microscopy (SEM, VEGA 3 XMU, Tescan, Czech Republic) equipped with secondary electron and back-scattered electron (BSE) detectors as well as with energy-dispersive X-ray spectrometer (EDS). Prior to observation, dry samples were mounted on a copper plate using carbon tape and sputter coated with a thin layer of Au–Pd (10 nm). The nHA incorporated into PLGA was examined by both BSE and EDS. The pore sizes of cane and biomorphic scaffolds were evaluated from their SEM micrographs by ImageJ software (National Institute of Health, USA). The crystalline phase of biomorphic scaffolds was analyzed by X-ray diffractometer (XRD, X’Pert PRO, PANalytical) applying monochromatic Cu Kα radiation. Data were collected over the range 2θ = 5–80°. The compression characteristics of biomorphic scaffolds along the channel direction were tested using a universal testing machine (Instron 5943, Instron Int. Ltd., USA), with a crosshead speed of 1 mm/min up to a maximum compression ratio of 65%. The compressive modulus was determined as the slope of the linear region of the stress–strain curve. Porosity was measured by Archimedes’ principle, using ethanol as displaced liquid. Measurements were done in triplicate, and the data reported represented means ± standard deviations.

2.4. Morphology of MC3T3-E1 cells on biomorphic scaffolds

MC3T3-E1 cells were maintained in culture flasks in DMEM supplemented with 10% FBS, 2 mmol/l l-glutamine, 100 units/ml penicillin and 0.1 mg/ml streptomycin, placed in a fully humidified incubator (37 °C, 5% CO2). To reveal the morphology of MC3T3-E1 cells on the biomorphic scaffolds, both PLGA and PLGA/nHA composite scaffolds were sterilized by soaking in 75% ethanol for 2 h, washed with phosphate buffered saline (PBS) and distilled water three times. 500 μl of MC3T3-E1 single cell suspension (density: ~1 × 105 cells/ml) was pipetted onto each scaffold placed in a 24-well culture plate. After 4 h post-seeding, 1 ml of culture medium was added to each well. The cell/scaffold constructs were incubated for up to 5 days. The culture medium was refreshed every day. At each selected time point, selected scaffold/cell constructs were washed twice with PBS and fixed with 2.5% glutaraldehyde at 4 °C overnight. After that, the constructs were dehydrated using a series of gradient ethanol solutions (from 30% to 100%) and dried by a critical point dryer (Quorum/Emitech K850, UK). The dry samples were sputter-coated with gold and observed by SEM.

2.5. Fluorescent staining

The viability and localization depth of MC3T3-E1 cells on the biomorphic PLGA/nHA scaffolds were assessed using FDA staining. Briefly, equal numbers of cells (104) were seeded onto each scaffold in 24-well plates, and the cell/scaffold constructs were incubated in a humidified atmosphere containing 5% CO2 at 37 °C. After 1 and 5 days of cultivation, the culture medium was replaced, and the MC3T3-E1 cells were washed with PBS and incubated in culture media containing FDA (5 μg/ml) for 20 min in the dark. The stained cells were immediately imaged using a TCS SP2 confocal laser scanning microscope (CLSM, Leica, Germany). A depth projection micrograph was constructed from the image sections of a cell/scaffold construct using ImageJ software. The sections were acquired from the scaffold surface to its inner in z-stack mode at 5 μm vertical steps. For staining of actin microfilament, the cultured cells were fixed in 4% paraformaldehyde and stained with phalloidin-FITC, and the cell nuclei were counter-stained using PI.

2.6. MTT assay

The proliferation of MC3T3-E1 cells on biomorphic scaffolds was estimated using MTT assay. MC3T3-E1 cells were seeded onto biomorphic...
PLGA and PLGA/nHA composite scaffolds in 24-well plates at a density of \(3.5 \times 10^4\) cells/well. After 1, 3 and 5 days of incubation, the culture medium was replaced with 100 μl of MTT solution (freshly made 5 mg MTT powder in 1 ml DMEM). Following incubation for 6 h at 37 °C in 5% CO₂, the MTT solution was removed, and 600 μl DMSO was added into each well to completely dissolve the precipitated formazan crystals. The formazan solution (100 μl) of each sample was subsequently added into individual wells of a 96-well plate and the absorbance at a wavelength of 490 nm was measured by a microplate reader (Bio-Tek Instruments Inc., USA). Tests were conducted in triplicate.

2.7. ALP activity assay

MC3T3-E1 cells were cultured on biomorphic scaffolds under the same conditions described above. Quantitative ALP activity was determined using a colorimetric assay based on hydrolysis of p-nitrophenyl phosphate substrate to p-nitrophenol [36]. After 1, 3 and 5 days of incubation, cell/scaffold constructs were washed with PBS, minced, incubated in lysis buffer and then centrifuged (1500 rpm) for 10 min to remove all insoluble debris. The ALP activity tests were performed according to the manufacturer’s instructions. Measurements were performed in triplicate.

2.8. Statistical analysis

Statistical analysis was performed using the SPSS 13.0 software. The analysis of variance (ANOVA) method was employed to measure the statistical difference between the results from the different groups at the same time point with a minimum confidence level of \(p < 0.05\) for statistical significance. All values were reported as mean ± standard deviation.

3. Results and discussion

3.1. Morphology of biomorphic scaffolds

Cane was selected as the starting template because it has similar porous structure and architecture to spongy bone. The SEM images of cane and the as-prepared biomorphic PLGA and PLGA/nHA composite scaffolds are shown in Fig. 1. As displayed in Fig. 1A and B, cane exhibited a unique hierarchically anisotropic cellular morphology which was characterized by highly interconnected honeycomb-like channels. The typical channel diameters were in the range of 80 to 220 μm. All the parallel channels were surrounded and interconnected by a lot of micropores (≤10 μm). The porosity of cane was 73.7 ± 0.7%. Such porous characteristics might meet the requirement for bone tissue engineering. The parallel channels could provide excellent accessibility for low

![Fig. 1. SEM images of cane (A, B), and biomorphic PLGA scaffold (C, D) and PLGA/nHA composite scaffold (E, F). SEM images of PLGA/nHA composite scaffold were obtained in the BSE mode.](image)
viscosity infiltration and leaching process, which guaranteed the smooth conversion of hierarchical porous structure of cane to polymer-based scaffolds. The SEM images in Fig. 1C–F demonstrated that both PLGA and PLGA/nHA composite scaffolds well preserved the original porous architecture of cane at the macro and submicron levels, which is in agreement with our hypotheses. The biomorphic scaffolds exhibited a pronounced bimodal pore size distribution with average channel diameter and micropore size of approximately 164 ± 52 μm and 13 ± 8 μm, respectively, which were consistent with those of cane, confirming the successful transformation.

The porosity of the biomorphic PLGA and PLGA/nHA scaffolds was 88.1 ± 1.7% and 89.3 ± 1.4%, respectively, which indicated that the incorporation of nHA into PLGA had a negligible effect on porosity. Such porous structure has been verified to effectively promote the transport of nutrients and oxygen and osseointegration [37,38]. It was also noted that the porosity of the biomorphic scaffolds was obviously higher than that of cane. The reason for this phenomenon is that initial woody pore struts were replaced by either PLGA solution or PLGA/nHA composite suspension during the transformation process, and that the removal of solvent left many pores that gave rise to an obvious increase in the total porosity. To visually evaluate the dispersion of nHA in PLGA, PLGA/nHA composite scaffolds were observed by SEM in the BSE mode. It could be seen from the high magnification BSE image of biomorphic PLGA/nHA composite scaffolds (Fig. 1F) that a great number of nanoscale white dots uniformly dispersed in PLGA matrix. The white dots were HA nanoparticles, as confirmed by energy-dispersive X-ray analysis (inset in Fig. 1F). The nanostructured surface morphology of the micropore struts, caused by nHA, would allow biological systems like cells to utilize the biomorphic scaffolds at the micro/nanometer scale. Therefore, the biomorphic PLGA/nHA composite scaffolds could provide a favorable substance for cell attachment and growth.

3.2 Phase structure and compression characteristics of biomorphic scaffolds

The phase structure and compression characteristics of the biomorphic scaffolds were characterized by XRD and static compression test techniques, and the results are shown in Figs. 2 and 3, respectively. As revealed in Fig. 2a, the XRD pattern of biomorphic PLGA scaffold showed a broad band with two narrow XRD peaks at 2θ = 16.6° and 22.3°, respectively, indicating the semi-crystalline nature of PLGA. In the XRD pattern of PLGA/nHA composite scaffold (Fig. 2b), the above-mentioned two peaks disappeared, and the typical sharp XRD peaks of nHA appeared. The result was consistent with the EDS data (Fig. 1F), which further confirmed the presence of nHA in PLGA. It could be concluded from the results that the incorporation of nHA into PLGA decreased the degree of crystallinity of PLGA.

Fig. 2. XRD patterns of biomorphic PLGA (a) and PLGA/nHA composite (b) scaffolds.

3.3 Morphology of MC3T3-E1 cells on biomorphic scaffolds

To evaluate the biocompatibility of biomorphic PLGA and PLGA/nHA composite scaffolds, the morphologies of MC3T3-E1 cells on the biomorphic scaffolds cultured for different periods were investigated by SEM, and the results are presented in Fig. 4. Fig. 4 displayed the distinct morphologies of MC3T3-E1 cells observed on the scaffolds after 1, 3 and 5 days of culture. It was seen that the MC3T3-E1 cells seemed to adhere to the biomorphic scaffolds and mainly settled at the micropores of channel walls. The SEM micrographs in Fig. 4A and D reflected the status of MC3T3-E1 cell attachment after 1 day of culture. The MC3T3-E1 cells on the biomorphic PLGA scaffolds exhibited a spherical morphology, while those on the biomorphic PLGA/nHA scaffolds showed spherical and polygonal morphologies. On both scaffolds, there were some filopodia-like structures anchoring the MC3T3-E1 cells, and the surfaces of the cell bodies displayed microvilli-like projections. After a cultivation period of 3 days, the MC3T3-E1 cells on the PLGA scaffolds became stretched out and exhibited a more filopodia-like structure. The cells were in the progress of spreading. In contrast, the MC3T3-E1 cells on the PLGA/nHA composite scaffolds displayed a more flattened, well-spread morphology. Some flattened cells anchored so tightly on the surface of the pore struts that it was difficult to distinguish cell–material interfaces. When culture time was prolonged to 5 days, there were more cells on the biomorphic PLGA/nHA composite scaffolds than the biomorphic PLGA scaffolds. The cells on the biomorphic PLGA/nHA scaffolds exhibited a well-spread morphology, and some neighboring cells directly contacted each other and formed a confluent monolayer, suggesting cellular proliferation and migration. However, the cells on the biomorphic PLGA scaffolds showed a poorly-spread, polygonal morphology. The results confirmed that the biomorphic PLGA/nHA scaffolds were favorable substance for cell attachment and growth.

Fig. 3. Representative stress–strain curves of biomorphic PLGA scaffold and biomorphic PLGA/nHA composite scaffolds (in dry and wet statuses).
composite scaffolds provided a favorable microenvironment for MC3T3-E1 cells, and better supported their attachment, spreading and growth than the biomorphic PLGA scaffolds. The morphological alterations of the MC3T3-E1 cells implied that the nHA incorporated into PLGA resulted in the nano-scaled rough surface and affected cell morphology by promoting the cellular adhesion and spreading [39].

By comparing the results of the current study with those obtained in our previous studies on biomorphic poly(γ-benzyl-L-glutamate) (PBLG) and poly(ε-caprolactone)/nHA (PCL/nHA) composite scaffolds [23,40], it could be found that the MC3T3-E1 cells on biomorphic PBLG scaffolds grew better than those on biomorphic PLGA scaffolds, but they did not grow so well as those on biomorphic PLGA/nHA composite scaffolds. The possible reasons for the results are as follows: (1) PBLG had a better hydrophilicity than hydrophobic PLGA, and (2) the incorporation of nHA into PLGA produced more favorable surface chemistry and nanostructured morphology for cell attachment and growth compared to the PBLG scaffolds. In addition, the MG-63 cells on biomorphic PCL/nHA composite scaffolds proliferated faster than the MC3T3-E1 cells on PLGA/nHA composite scaffolds under the similar conditions.

3.4. Fluorescent staining analysis

To further explore the attachment, viability and proliferation of MC3T3-E1 cells on the biomorphic PLGA/nHA composite scaffolds, FDA staining and immunofluorescence staining were carried out, and the corresponding CLSM images are given in Figs. 5 and 6, respectively. As shown in Fig. 5A and B, the FDA staining images indicated that live MC3T3-E1 cells (green) dispersed homogeneously on the channel walls, and the number of cells strongly depended on the culture time. This implied that the MC3T3-E1 cells had greatly proliferated on the biomorphic PLGA/nHA composite scaffolds. To detect the localization depth and viability of the cells in the biomorphic PLGA/nHA composite scaffold on day 5 in culture, the cell/scaffold construct was investigated by CLSM z-stacks. The projected z-stacks presented in Fig. 5C clearly showed that the cells were capable of attaching onto the deep sections of the scaffolds. Some cells could localize up to approximately 400 μm within the channels, which was larger than the penetration depth of osteoblasts on PLGA foam scaffolds [41]. The possible reason for the result is that the parallel channels within the biomorphic scaffolds facilitated both cell seeding and oxygen/nutrient exchanges.

The immunofluorescence staining images in Fig. 6 confirmed that the MC3T3-E1 cells showed good attachment, spreading and growth. After 1 day post-seed, a majority of MC3T3-E1 cells uniformly existed on the channel walls as scattered single cells, and some cell clusters appeared. In contrast, the cells of day 5 culture displayed a good filamentous, stretched morphology, which indicated that the F-actin was well developed. This was significantly different from the spherical morphology of the cells after 1 day of culture. Some cells bridged across the channels, and even formed a confluent cell monolayer covering some channels, which was common on the whole scaffold surface. Similar cell behavior was also observed in the honeycomb-like polycaprolactone-based scaffold manufactured using rapid prototyping technique [42].
3.5. Proliferation and differentiation of MC3T3-E1 cells on biomorphic scaffolds

The proliferation of MC3T3-E1 cells cultured on the biomorphic scaffolds over a 5-day culture period was quantitatively investigated by MTT assay, and the results are shown in Fig. 7. As displayed in Fig. 7, the metabolic activity (optical density (OD) value) of the MC3T3-E1 cells on both scaffolds increased over the 5 days culture period, and the OD value for the biomorphic PLGA/nHA composite scaffolds was higher than that for the biomorphic PLGA scaffolds at each time point. The cell metabolic activity matched the SEM results in Fig. 4. The results demonstrated the proliferation of MC3T3-E1 cells on both scaffolds, because the higher OD value resulted from a larger number of live cells or metabolizing cells [43]. It was also noted that there was no significant difference in OD value between the biomorphic PLGA and PLGA/nHA composite scaffolds (p > 0.05) after 1 day of culture, whereas the OD values for the biomorphic PLGA/nHA composite scaffolds were significantly higher than those for the biomorphic PLGA scaffolds on day 3 and day 5 (p < 0.05). The results indicated that the nHA incorporated into PLGA could stimulate cell proliferation. The possible reason for this is that nHA improved the surface roughness of pore struts and interfered with the attachment and growth behaviors of MC3T3-E1 cells.

The osteogenic differentiation of MC3T3-E1 cells cultured on the biomorphic PLGA and PLGA/nHA composite scaffolds was also evaluated by quantifying ALP activity, and the levels of ALP activity are shown in Fig. 8. It was found that the ALP activity of MC3T3-E1 cells cultured on both scaffolds increased with the increasing culture time, and the ALP activity on the biomorphic PLGA/nHA composite scaffolds increased more significantly. This is expected because ALP is generally considered as an early biomarker for osteoblastic differentiation of pre-osteoblasts [44,45]. A higher ALP activity reflected to a more differentiated stage towards mature osteoblasts. Low ALP activity could be detected after 1-day or 3-day culture, but no significant difference in the ALP activity was observed between the biomorphic PLGA and PLGA/nHA composite scaffolds (p > 0.05). On day 5 post-seeding, the MC3T3-E1 cells grown on the biomorphic PLGA/nHA composite scaffolds exhibited a much higher level of ALP activity than those on the biomorphic PLGA scaffolds (p < 0.05), possibly due to the presence of nHA which is widely known to induce in vitro osteoblastic differentiation of progenitor cells towards osteoblasts [44,46,47]. The results indicated that the biomorphic PLGA/nHA composite scaffolds could support the osteogenic differentiation of pre-osteoblastic MC3T3-E1 cells into mature osteoblasts.

4. Conclusions

Biomorphic PLGA/nHA composite scaffolds mimicking the ECM of sponge bone were successfully fabricated through a two-step transformation process using cane as a template. The biomorphic PLGA/nHA
composite scaffolds well preserved the morphology and cellular microstructure of cane at the micrometer scale, and had a bimodal pore size distribution (164 ± 52 µm and 13 ± 8 µm) with a high porosity of 89.3 ± 1.4%. The incorporation of 5 wt.% nHA into PLGA decreased the degree of crystallinity of PLGA, and significantly improved the compressive modulus of biomorphic scaffolds from approximately 539.3 ± 36.2 kPa to 1354.6 ± 53.7 kPa. The compressive modulus of wet PLGA/nHA composite scaffolds was slightly lower than that of dry scaffolds. Compared to the biomorphic PLGA scaffolds, the biomorphic PLGA/nHA composite scaffolds could better support the attachment, proliferation and osteogenic differentiation of MC3T3-E1 cells. Collectively, our results demonstrated that the novel biomimetic PLGA/nHA composite scaffolds were promising for bone tissue engineering applications.

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