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Original Article

Evaluation of poly-ε-caprolactone/graphene oxide three-dimensional porous scaffolds for potential application in alveolar bone repair

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KEYWORDS
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Alveolar bone

Abstract *Background/purpose:* Alveolar bone reconstruction or regeneration demands the interim porous scaffold. It possesses adequate mechanical properties to enable it to withstand injured bones. These scaffolds are used to regulate the cell growth that migrate from surrounding tissue or are implanted within porous scaffold. This study is devoted to the manufacturing of 3D porous scaffolds and application on repair and regeneration of alveolar bone.
Materials and methods: Graphene oxide (GO) was mixed with poly-ε-caprolactone (PCL) material to fabricate 3D porous scaffolds by a solvent-casting/particulate-leaching method. The effects of various concentrations of GO (0.05, 0.1, 0.5, 1, and 2 wt.%) in PCL/GO scaffolds were focused on biological and physical properties. The human osteosarcoma cell (MG-63) in vitro was determined the biocompatibility of PCL/GO scaffolds.

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Results: The PCL/GO scaffolds had the large porosity (greater than 88 %) in this study ($P < 0.05$). The Young's modulus of PCL/GO scaffold matched with human alveolar cancellous bone and it could be employed as the repair and support on this bone. The degradation rate of PCL/GO scaffolds was much lower than that of PCL scaffolds. The MG-63 cell displayed excellent attachment and proliferation on the PCL/GO scaffolds.
Conclusion: The 3D porous scaffold had an interconnected structure and its pore diameter was from 250 to 400 μm. Graphene oxide changes the surface properties of the 3D porous scaffold from hydrophobic to hydrophilic. The ALP assay indicated that MG-63 cell differentiated better in PCL/GO scaffolds containing 1 wt.% GO than on other scaffolds.

Introduction

The alveolar bone reconstruction or regeneration often demands the interim scaffold with porous pores. The porous scaffolds needed to possess sufficient mechanical properties to enable them to withstand bone injury. These kind scaffolds are often used to regulate cell growth that migrate from surrounding tissue or are seeded within the pores of porous scaffold to solve the problem of alveolar bone repair in clinical practice. There are many papers focused on the various polymers plus hard materials (hyaluronic acid, graphene, and graphene oxide) to formed the scaffolds to enhance the scaffold's mechanical property and did cell culture to explore the biocompatibility of scaffolds.[1–7] The growth of bone tissue and vascular tissue required the pore size of scaffold greater than 100 μm, while a scaffold's pore size of 100—350 μm is suitable for bone formation.[8]

Graphene-based materials (graphene, graphene oxide (GO), and reduced graphene oxide (rGO)) are rapidly emerging materials for scaffolds due to both their unique structure and their excellent mechanical, electrical, and optical properties. Various materials based on graphene oxide have been widely used in electronic and other fields. In recent years, these special properties have been used in the biomedical field, especially in the field of tissue engineering. The mechanical properties and cell culture of polymer/GO and polymer/rGO scaffolds in tissue engineering to help alveolar bone repair.[9–19] GO scaffolds in dental applications such as alveolar bone healing and biological characteristics of human dental pulp stem cells.[20,21]

The human alveolar bone and other bones in the body are composite materials (flexible enough to resist bending and strong enough to withstand external impact).

This study simulated this situation, using PCL plastic as an elastic material and graphene oxide as a rigid material to create a biomedical scaffold to repair alveolar bone damage. In this study, graphene oxide (GO) platelets were mixed with PCL solution to fabricate 3D porous scaffolds using a solvent-casting/particulate-leaching process. The extracellular matrix of natural human bone contains the complex organic-inorganic composite materials. Poly ε-caprolactone (PCL) is the semi-crystalline material that is one of the most widely available biodegradable polymers based on its toughness, biocompatibility, and cost-effectiveness. However, its poor surface wettability and hydrophobicity cause some problems in cell adhesion and proliferation and its mechanical properties also restrict its use. The effects of GO on the physical properties, mechanical properties and degradability of PCL/GO scaffolds are studied. The clinical application of this study is to repair the alveolar bone when it is damaged.

Materials and methods

The 3D porous poly-ε-caprolactone (PCL)/graphene oxide (GO) scaffolds were fabricated by a solvent-casting/particulate-leaching method. The PCL acted the matrix material (Mn: 80,000, Sigma—Aldrich, St. Louis, MO, USA). The porogen made use of NaCl (Taiyen, Tainan, Taiwan). GO platelets were prepared by transferring the graphite intercalation compound into a preheated crucible at 700 °C in a common furnace that was placed in the front of a fume cupboard to prevent inhalation of the nanoparticles, and it was left there for 60 s. These layers expanded upon ultrasonication, and caused the GOs to disperse in the solvent and GO was fabricated by the authors (Fig. 1). Revealed the fabrication process of the PCL/GO scaffold. The PCL/GO scaffolds were prepared by added various percentage GO (0.05, 0.1, 0.5, 1, and 2 wt.% GO, respectively). Raman spectroscopy was applied to analyze the PCL/GO scaffolds.

Archimedes' principle applied on porosity of PCL/GO scaffolds:[22] where Ws: scaffold weight, Wa: weight of bottle with ethanol, Wb: weight of bottle with ethanol and scaffold, Wc: weight of bottle with ethanol (excluding the saturation scaffold), ρs: PCL density, and ρe: ethanol density.

$$\phi_p(\%) = \frac{(W_b - W_c - W_s)/\rho_e}{(W_a - W_c)/\rho_e + W_s/\rho_s} \quad 1$$

This study analyzed the hydrophobicity or hydrophilicity of PCL/GO scaffolds by contact angle measurement. The surface morphology of PCL/GO scaffolds was measured by a scanning electron microscope (TM3030, Hitachi, Tokyo, Japan).

The mechanical properties of PCL/GO scaffolds were evaluated by a texture analyzer (Texture Technologies,

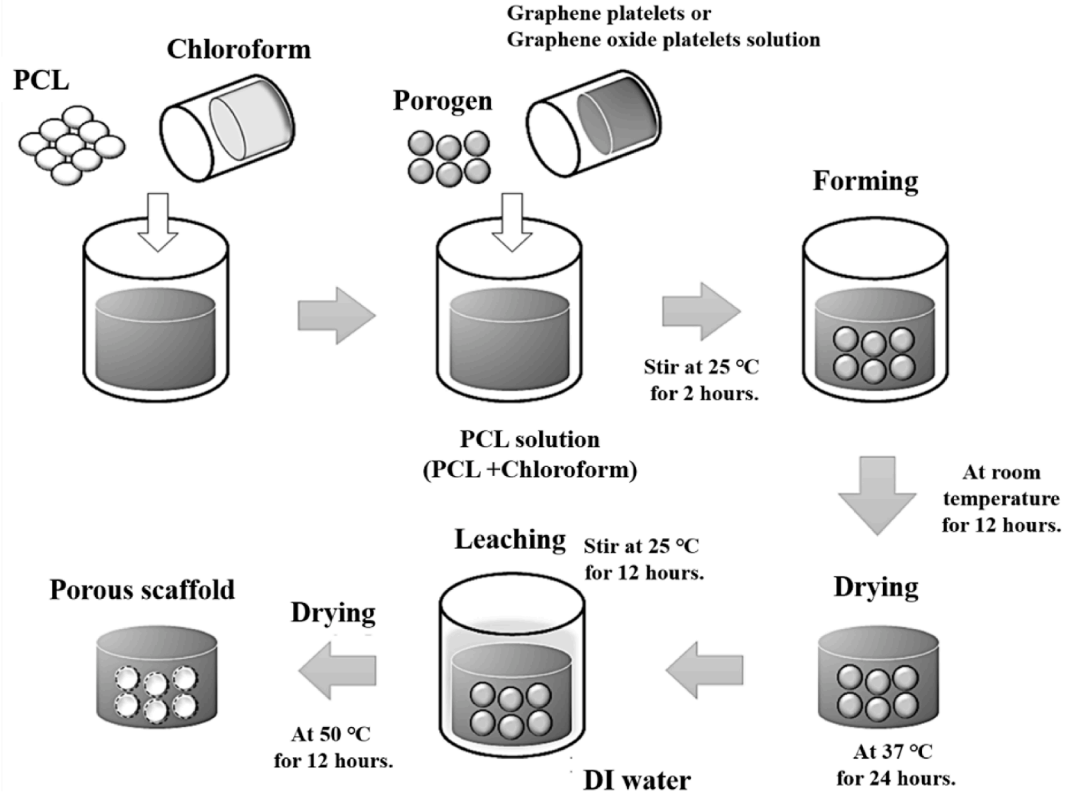

Figure 1 Preparation schedule of the poly-ε-caprolactone/graphene oxide porous scaffolds. PCL: poly-ε-caprolactone. DI water: deionized water.

Hamilton, MA, USA).[23,24] The compressive strength of PCL/GO scaffolds by formula 2.σ: compressive strength, F: maximum load, A: cross-sectional area of scaffold, and D: scaffold diameter.

$$\sigma = \frac{F}{A} = \frac{F}{\pi D^2/4} \quad 2$$

The theoretical compressive strength was coming from formula 3.[25] σc: compressive strength, ϕp: scaffold porosity (only use numerical values, not percentages.).

$$\sigma c(\phi p) = 700 \exp(-5\phi p) \quad 3$$

The degradation test used to investigate the degradation rate and pH values of the 3D porous scaffolds. The 3D porous scaffolds were degraded in phosphate-buffered saline (PBS) at 37 °C. The pH value of degraded PBS was measured using a pH meter (Model 710-2, Orion, Thermo Fisher Scientific, Waltham, MA, USA).

The sample's weight loss (Wloss) is calculated as formula 4. Wori: sample weight before degradation, and Wdeg: sample weight after degradation.

$$W_{loss} = \frac{W_{ori} - W_{deg}}{W_{ori}} \times 100\% \quad 4$$

The human osteosarcoma cell (MG-63) were seeded in PCL/GO scaffolds at different GO percentage to evaluate cell growth behavior. Cells were cultured in a cell culture incubator at 37 °C and 5 % CO_2, and the culture medium was updated every 3 days. Immersed the samples in 95 % ethanol overnight for sterilization and then washed twice using PBS (Thermo Fisher Scientific) to remove residual ethanol. Then, the samples were transferred to a 24-well plate. The cells were detached by 0.25 % trypsin–EDTA (Thermo Fisher Scientific), each sample was transferred to a 24-well plate, and 0.5 ml of cell suspension was seeded at a concentration of 1×10^4 cells/ml. In this study, samples were taken out on days 1, 7 and 14 to observe the results of cell culture. The scaffolds inspected the cell culture's results on days 1, 7 and 14. The 3-(4,5-dimethylthiazole-2-Y1)-2,5-diphenyltetrazolium bromide (MTT) and alkaline phosphatase (ALP) assays were performed to gain the qualitative and quantitative results on cell culture. An enzyme-linked immunosorbent assay (ELISA) reader applied to measure the spectrophotometric absorbance at 405–690 nm.

All experiment data are presented as the mean ± standard error (SE) for each group of samples. All experiments had at least three scientific replicates. Data were analyzed using SPSS version 15.0 statistical software (SPSS, IBM, Chicago, IL, USA). A one-way analysis of variance (ANOVA) and t-test method were used to determine relevant differences in data. Significance levels were at $P < 0.05$.

Results

The pore size of PCL/GO scaffolds ranged from 250 to 400 μm (Fig. 2). PCL scaffold's porosity was 90.10 %. The PCL/GO scaffold containing 1 wt.% GO added has the maximum porosity (Fig. 3). The addition of GO resulted in

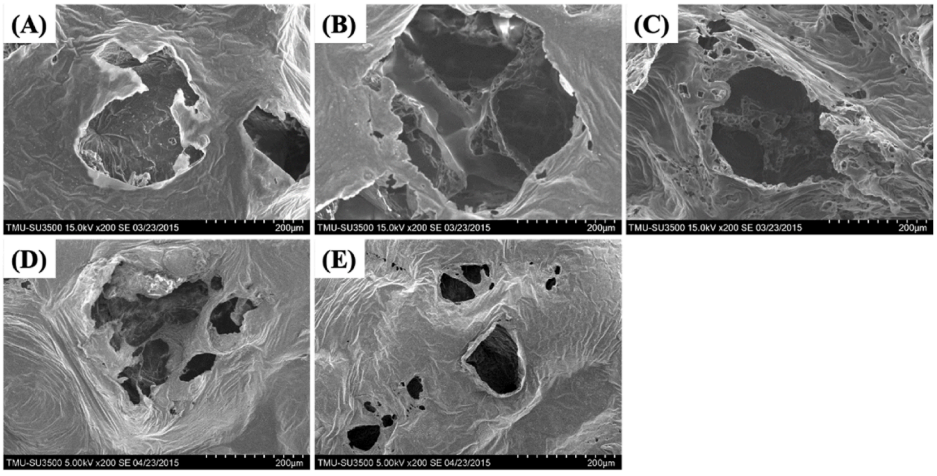

Figure 2 The SEM images of the porous structure of PCL/graphene oxide scaffolds with various percentages of graphene oxide platelets. (A) 0.05 wt.% GO. (B) 0.1 wt.% GO. (C) 0.5 wt.% GO. (D) 1.0 wt.% GO. (E) 2.0 wt.% GO.

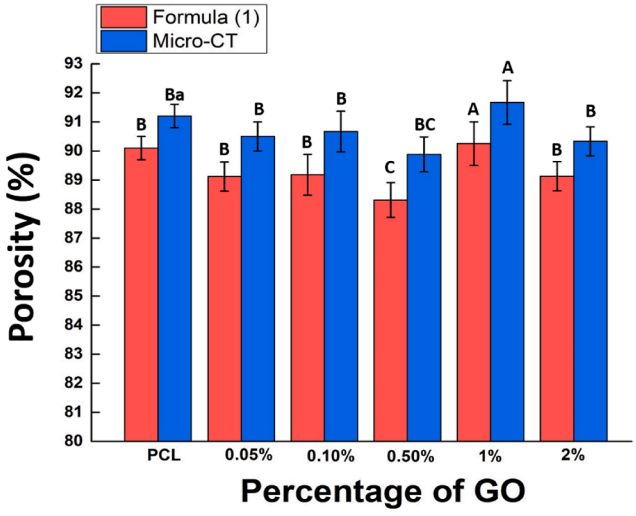

Figure 3 Porosities of the PCL and PCL/graphene oxide scaffolds with graphene oxide platelets of various percentages. The significant difference ($P < 0.05$) was determined by one-way analysis of variance (ANOVA). Different capital letters indicate differences between groups. PCL: poly-ε-caprolactone. GO: graphene oxide.

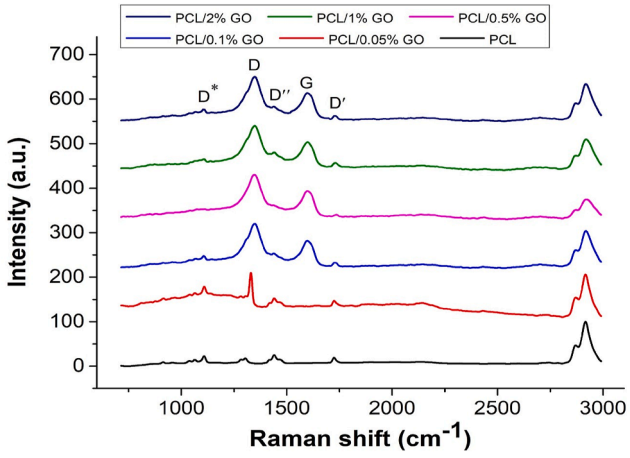

Figure 4 Raman spectroscopy of PCL/GO scaffolds with various percentage of GO. PCL: poly-ε-caprolactone. GO: graphene oxide.

increase of D, G, D', D", and D* peaks (Fig. 4). The Raman spectrum of GO has five peaks at 1330-1350 cm^{-1} (D), 1585 cm^{-1}(G), 1620 cm^{-1}(D'), 1500-1550 cm^{-1}(D") and 1150-1200 cm^{-1}(D∗). The above results are consistent with the literature.[26,27]

The PCL scaffold's contact angle was 112.5° (hydrophobicity) (Fig. 5A). The contact angles of PCL/GO scaffolds added different percentage GO were smaller than 90° (hydrophilicity). The PCL/GO scaffold has the smaller contact angle with 1 and 2 wt.% GO added. The PCL scaffold's compressive strength was 0.53 MPa (Fig. 5B). The compressive strength improved as increasing the GO percentage. The compressive strength of PCL/GO scaffolds containing 2 wt.% GO exceeded that of the other scaffolds. The experimental/theoretical compressive strengths of PCL/GO scaffold with various GO ratios added are very similar.[6,28] The Young's modulus of PCL/GO scaffolds (0.26—0.79 GPa) matched that of human cancellous bone (0.05—0.5 GPa).[29]

There was no rapid change for the degradation rate on PCL/GO scaffolds containing various percentage GO (Fig. 6A). The PCL/GO scaffolds containing higher weight ratios (0.5, 1, and 2 wt.%) of GO were more effective in reducing weight after 10 weeks. The weight loss of PCL/GO scaffold with added 1 wt.% of GO has the minimum value. The decrease in pH value is due to the generation of carboxylic acids during PCL materials' degradation (Fig. 6B).[28] The pH of PCL scaffold was 6.8 after 15 weeks. The PCL/GO scaffold with 1 wt.% GO added has the largest pH after the 15 weeks.

As the cell culture time increased, the number of viable cells increased, which demonstrated the excellent biocompatibility of PCL/GO scaffold (Fig. 6B).

The OD values of PCL/GO scaffolds with different percentages of GO did not change significantly at the 1st day.

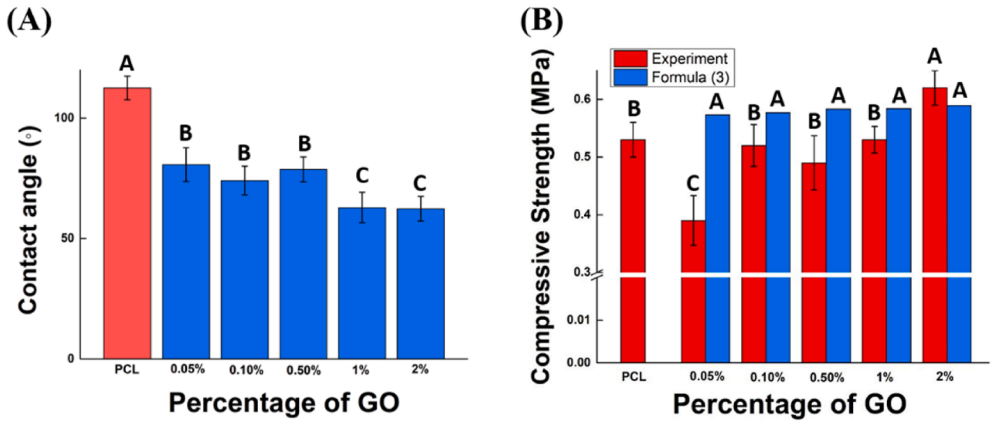

Figure 5 Physical and mechanical properties of PCL/graphene oxide scaffolds. (A) contact angles. (B) Compressive strengths. The significant difference ($P < 0.05$) was determined by one-way analysis of variance (ANOVA). Different capital letters indicate differences between groups. PCL: poly-ε-caprolactone. GO: graphene oxide.

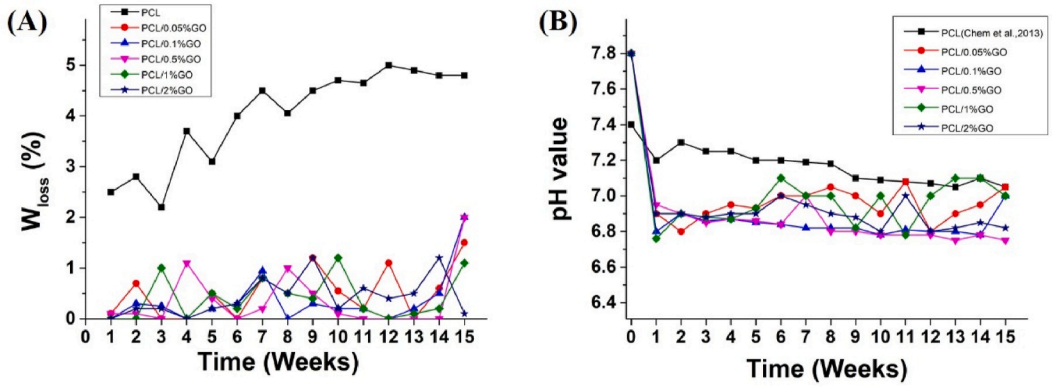

Figure 6 Degradation property analysis of PCL/graphene oxide scaffolds. (A) weight loss. (B) pH values. PCL: poly-ε-caprolactone. GO: graphene oxide.

The scaffolds' OD values with 1.0 and 2.0 wt.% GO addition were greater than those of other scaffolds and showed obvious differences from the scaffolds with 2 wt.% GO addition after the 7th day. The OD value of PCL/GO scaffold incorporating 1.0 wt.% GO was larger than that of other scaffolds, and was significantly different from the scaffold incorporating 0.05 wt.% GO on the 14th day. The ALP's concentration was converted from the ALP standard curve (Fig. 7). The two sets of data show the same trend. The ALP expression was low and no significant differences were detected among all PCL/GO scaffolds on day 1. No significant differences in ALP expression were detected between all PCL/GO scaffolds at day 7. The PCL/GO scaffolds containing 1.0 wt.% GO had significantly higher ALP activity than the other scaffolds and showed the most cell differentiation at day 14. Furthermore, the ALP activity of PCL/GO scaffolds containing 1.0 wt.% GO was significantly different compared with other scaffolds at day 14. The cell differentiation was most obvious in the PCL/GO scaffolds containing 1.0 wt.% GO added.

Discussion

Alveolar bone reconstruction or regeneration demands the interim scaffolds with the porous structures. The porosities and pore sizes of 3D porous scaffolds influence the ingress of nutrients and the egress of waste products. Pores that are too small or too large will obstruct cell growth, and each cell has its most suitable optimal pore size. The micron-scale connected pore structure will ensure smooth tissue growth and nutrient delivery.[8] The highly porous structures of PCL/GO scaffolds were easy to interact with cells. Scaffolds with larger pore sizes may not only provide gas diffusion and nutrient supply, but may also result in poor cell attachment. Small pores could facilitate cell attachment but may also hinder the transport of nutrients and gases.[30] The pore diameter of PCL/GO scaffold was from 250 to 400 μm. The pore sizes of 100–350 μm were generally considered necessary on alveolar bone regeneration and osteo-conduction.[8,31,32] The porosity of all PCL/GO scaffold overstepped 80 %, making them suitable on tissue engineering.[33]

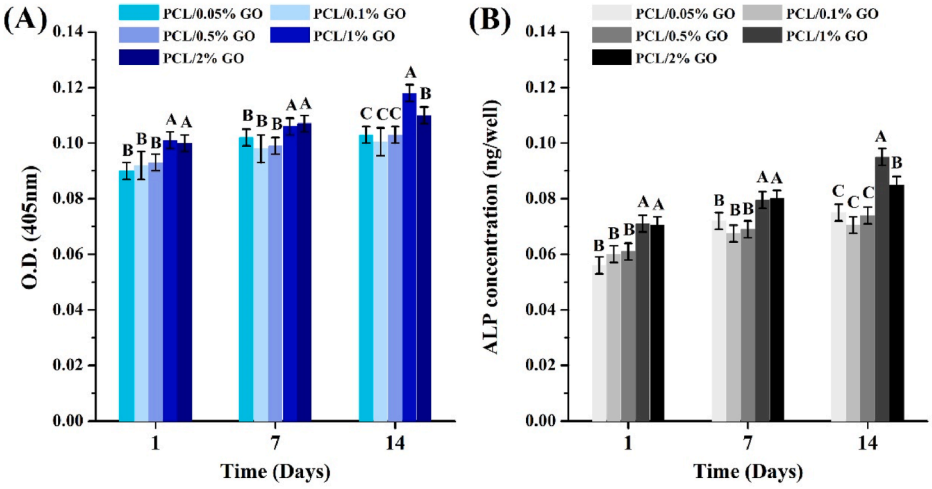

Figure 7 Biocompatibility testing of PCL/graphene oxide scaffolds. (A) Cell viabilities for MG-63 cells cultured on scaffolds. (B) ALP activities for MG-63 cells cultured on scaffolds. The significant difference ($P < 0.05$) was determined by one-way analysis of variance (ANOVA). Different capital letters indicate differences between groups. PCL: poly-ε-caprolactone. GO: graphene oxide.

The edge effect of GO due to aromatic structural disorder or oxidation is related to the D peak, while the G peak is caused by C—C bond stretching. The D band is due to defects and disorder in carbon lattice and double resonance processes near K point at Brillouin zone boundary. The G band is relative to the Raman-allowed E_{2g} (E_{2g} is the scattering mode in Raman spectroscopy. E_{2g} is the phonon vibration mode in nanocrystals and has a significant peak in Raman spectroscopy. optical phonons. The D′ band is the intravalley resonance corresponding to G band, and it is split by impurities. The D″ band is associated with amorphous phase, and its intensity is inversely proportional to crystallinity. D∗ band is known to originate from the sp3 orbitals. The five peaks of Raman spectra (D, G, D', D", and D∗) of GO were similar to existing literature results.[26,27] Raman spectral allows the assessment of covalent bonding or disorder. Intensity ratio of D band to G band (ID/IG) identifies defects in carbon materials. The ID/IG values of PCL/GO scaffolds with 0.05, 0.1, 0.5, 1, and 2 wt.% GO added were 1.567, 1.568, 1.569, 1.573, and 1.630. The ID/IG increased slightly with increasing GO concentration. This ratio represented the success of covalent bonding of GO to oxygen-containing groups.[26] this leaded to introduction of a large number of defects. Covalent bonds are generated between free radicals (salt) and C—C bonds of GO. When salt was heated, free radicals of highly active would be generated to attack the sp^2 carbon atoms of GO and form covalent bonds. The degree of covalent functionalization uses the ID/IG.[28,29] Additionally, the PCL/GO scaffold's defects leaded to increased oxygen content. The more oxygen-containing functional groups there were on material surface, the better its hydrophilicity was, which had a significant effect on enhancing cell vitality. The GO usually has 40 % oxygen groups, including OH, COOH and epoxy groups. This property makes it hydrophilic. Therefore, the GO addition enhanced the hydrophilicity of PCL/GO scaffold. The PCL/GO scaffolds were more hydrophilic than PCL scaffolds, which is similar to previous studies.[28,34]

The theoretical compressive strength of PCL/GO scaffold was decreased as the scaffold's porosity increased. The compressive strengths of PCL/GO scaffold through experiment were not much different from their theoretically value. The compressive strengths of PCL/GO scaffolds fabricated by experiment did not decrease with the increase porosity. The reason was that we used the solvent-casting/particulate-leaching method to prepare PCL/GO scaffold. The scaffold's GO could not be evenly distributed in PCL solution. Due to the uneven distribution of GO in chloroform, the addition of 2 wt.% GO may affect the matrix structure. Therefore, the compressive strengths of PCL/GO scaffold by solvent-casting/particulate-leaching method could not be related to the scaffold porosity. The Young's modulus of GO is 207.6—223.4 GPa, so it can enhance the compressive strength of PCL/GO scaffolds. The Young's modulus of PCL/GO scaffolds (0.26—0.79 GPa) matched Young's modulus of human cancellous bone (0.05—0.5 GPa). Therefore, the PCL/GO scaffold can be used to repair and support cancellous bone.

The degradation rate of all scaffolds increased slowly during the weight loss of the experiment. The degradation rate of PCL/GO scaffolds is lower than that of PCL scaffolds. GO usually contains various oxygen-containing functional groups (including epoxy, carbonyl, hydrocarbon, hydride, etc.), which can provide connection sites with various biological molecules such as proteins and DNA. By adding higher weight ratio GO to the scaffold, better cell viability was observed. The above results could be explained by previous papers,[9] which showed GO activated apoptosis but did not induce necrosis in U118 glioma cells. The MG-63 cells are an osteosarcoma-derived cell line. GO appeared to act as the same role in cell viability of MG-63 cells. The ALP concentration of PCL/GO scaffold containing 1 wt.% GO (0.095 ng/well) appeared significantly different compared with other scaffolds at day 14. The cell differentiation was most obvious on PCL/GO scaffolds containing 1 wt.% GO.[32]

The biocompatibility of PCL/GO scaffold was confirmed on MTT and ALP after cell adhesion. The MG-63 cells grow well on PCL/GO scaffolds, revealing that PCL/GO scaffold was more suitable on cell culture. The feature of this study is to explore the effects of different weight ratios of GO on the physical and biological properties of PCL/GO scaffolds. The Young's modulus of PCL/GO scaffold obtained in this study matched that of human cancellous bone and this scaffold can be used as the repair and support on cancellous bone. The unique feature of this study is the incorporation of GO into PCL to form a composite scaffold, characterized by a Young's modulus close to that of alveolar bone. Compared to other studies that incorporate different 2D materials (such as carbon nanotubes), this composite scaffold is stronger and more suitable for alveolar bone regeneration and repair.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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