RESEARCH ARTICLE

3D-Printed scaffolds based on poly(Trimethylene carbonate), poly(ε-Caprolactone), and β-Tricalcium phosphate

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Abstract

Three-dimensional (3D)-printed scaffolds of biodegradable polymers have been increasingly applied in bone repair and regeneration, which helps avoid the second surgery. PTMC/PCL/TCP composites were made using poly(trimethylene carbonate), poly(ε-caprolactone), and β-tricalcium phosphate. PTMC/PCL/TCP scaffolds were manufactured using a biological 3D printing technique. Furthermore, the properties of PTMC/PCL/TCP scaffolds, such as biodegradation, mechanic properties, drug release, cell cytotoxicity, cell proliferation, and bone repairing capacity, were evaluated. We showed that PTMC/PCL/TCP scaffolds had low cytotoxicity and good biocompatibility, and they also enhanced the proliferation of osteoblast MC3T3-E1 and rBMSC cell lines, which demonstrated improved adhesion, penetration, and proliferation. Moreover, PTMC/PCL/TCP scaffolds can enhance bone induction and regeneration, indicating that they can be used to repair bone defects in vivo.

Keywords: Biodegradability; Bone regeneration; Cell proliferation; Poly(trimethylene carbonate); Poly(ε-caprolactone); β-tricalcium phosphate.

1. Introduction

In recent years, three-dimensional (3D)-printed scaffolds of biomaterials have been increasingly applied for bone regeneration and repairing of bone defects due to the advantage of individual structures1-3. The ideal biomaterials for bone regeneration scaffolds should have the characteristics, including good biocompatibility, good biodegradability, low toxicity, good mechanical properties, and ease of shaping and disinfection. Moreover, they should have appropriate porosity and pore size to provide a good environment for the growth of new bone tissue, and should have good bone conduction to form autogenous bone instead of substitutes4-7.

Biodegradable polymeric scaffolds can create the microenvironment and pore network milieu for cell adhesion and bone regeneration, allowing bone defects to be repaired without the need for the second surgery. Poly(ε-caprolactone) (PCL)8-9,
poly(trimethyl carbonate) (PTMC) [10,11] and poly(lactic acid) (PLA) [12-14] are some of the appealing biodegradable materials, which are exploited as the carriers for bioengineering and medication delivery. Due to its considerable biodegradability, biocompatibility, and mechanical property, PCL is suitable to be used for making biodegradable scaffolds for bone tissue engineering with minimal inflammatory impact of degradation compounds. However, PCL appears to be hard and have the bulk degradability, high hydrophobicity, and crisp characteristics [15-17]. Meanwhile, PTMC has good flexibility, good biocompatibility and biodegradability, low toxicity, and surface corrosion property. Therefore, it is one of the preferred synthetic thermoplastic biomedical polymers for manufacturing in the scaffold for bone regeneration. However, its weak mechanical strength generally hampers its wide application [18,19]. PTMC possesses certain good properties that can complement and overcome the disadvantages of PCL to improve the overall flexibility and degradation of the scaffolds.

β-Tricalcium phosphate (TCP) has been applied as an ideal bone guide material in orthopedics, spine, plastic surgery, and other fields. Its properties, such as good biocompatibility and biodegradation, excellent safety, and bone-guided regeneration ability, have been recognized by medical practitioners. TCP interacts with osteoblasts and is catabolized to participate in the construction of new bone through humoral dissolution and cell degradation after the implantation in vivo. Moreover, its other byproducts can enter the bloodstream and can be metabolized and expelled out of the body; therefore, it will not cause any damages to organs or tissues and give rise to pathological calcification [20-24].

The polymeric composites with nanoceramics offer more mechanical qualities due to the dispersion of nanoparticles in polymer matrix [25,26]. In this study, PCL was chosen as a hard polymer matrix and further modified by PTMC, which acted as a soft polymer plasticizer, while TCP nanomaterials were used as reinforcing fillers and bone regeneration inducer. PTMC/PCL/TCP composites in the present study were made of PTMC, PCL, and TCP. Following that, biodegradable PTMC/PCL/TCP scaffolds were created by utilizing biological 3D printing approach, and their qualities in bone implantation applications were also investigated.

2. Materials and methods

2.1. Preparation of PTMC/PCL/TCP scaffolds

PTMC/TCP composites were made of PTMC (100 – 75% mass) and TCP (0 – 25% mass), and PCL/TCP composites were made of PCL (100 – 75% mass) and TCP (0 – 25% mass). PTMC/PCL/TCP composites were made of PTMC (50 – 37.5% mass), PCL (50 – 37.5% mass), and TCP (0 – 25% mass) [27,28]. PTMC, PCL, and TCP were mixed in dichloromethane and stirred to ensure that the mixture was uniformly fixed. Subsequently, the mixtures were dried in vacuo to produce PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP composites.

PTMC/TCP, PCL/TCP, and PTMC/PCL/TCP scaffolds (diameter [mm] × height [mm]: 6 × 2, 10 × 2, and 4 × 6, respectively) were produced using Regenovo 3D Bio-Architect® Pro Biological 3D printer, and the above composites are used as the raw materials. The following printing conditions were adopted: Conducted at room temperature; inner diameter of print head ≥ 0.41 mm; extrusion pressure at 0.3 MPa; and printing rate at 6 mm/s [31].

2.2. Cell viability assay

The osteoblast cell lines, clonal murine cell line of immature osteoblasts derived from mice (MC3T3-E1) and rat bone marrow mesenchymal stem cells (rBMSCs), were provided by the China Center for Type Culture Collection of Wuhan University, China [29]. 3D-printed PTMC, PCL, PTMC/TCP, PCL/TCP, and PTMC/PCL/TCP scaffolds (diameter [mm] × height [mm]: 6 × 2; different TCP content: 0 – 25%) were sanitized for 1 h with ultraviolet light and fastened in 24-well plate. MC3T3-E1 cells (4 × 10^4, 200 µL) were seeded onto the scaffolds and cultured in the RPMI-1640 media in an incubator (37°C, 5% CO_2) for 1 and 3 days before being replaced with fresh growth medium. After that, cell counting kit 8 (CCK-8, 10 µL) solution, which was purchased from Solarbio (Beijing, China), was added into each well and incubated for 3 h. The absorbance (OD_{450}) was determined using a DG-3022A ELISA-Reader (iMark, BioRad, USA).

2.3. Degradation assay

All scaffolds (diameter [mm] × height [mm]: 10 × 2) were sealed in a dialysis bag containing phosphate-buffered saline (PBS, 10 mL) and shaken in PBS (pH 7.4, 90 mL) at 37°C. The samples were taken out every month, washed, and dried in vacuo. Following that, the samples’ molecular weight (M_w) was measured by gel permeation chromatography, and the weight loss was determined.

2.4. DOX release assay

5-DOX-Incorporated PTMC, PCL, PTMC/TCP, PCL/TCP, and PTMC/PCL/TCP scaffolds were prepared according to protocols described in previously published articles [13]. The samples sealed in a dialysis bag with PBS (10 mL) and shaken slowly in PBS (pH 7.4, 90 mL) at 37°C. At different time points, fresh PBS (25 mL) was replaced with dialysis solution (25 mL). The variations of DOX concentrations were analyzed.
2.5. In vitro biocompatibility assay

3D-printed PTMC, PTMC/TCP, PCL/TCP, and PTMC/PCL/TCP scaffolds with 25% TCP content (diameter [mm] × height [mm]; 6 × 2) were sterilized and placed into the 24-well plate. MC3T3-E1 cells (6 × 10^4, 200 μL culture medium) were seeded onto the scaffolds and cultured for 1 day. Subsequently, paraformaldehyde (4%, 1 mL) was added for 10 min and washed. The scaffolds were then stained using fluorescein isothiocyanate (FITC)-labeled phalloidin (500 nmol/L, Invitrogen Co., USA) for 30 min and washed. Cells were stained again with 4',6-diamidino-2-phenylindole (100 nmol/L, Invitrogen Co., USA) for 10 min and washed, and the scaffolds were stored or photographed at 4°C. The adhesion and growth of the cells in scaffolds were observed using a fluorescence inverted microscope (IX-70, Olympus Co., Ltd., Japan).

2.6. Osteogenic gene expression

rBMSCs (initial density: 8 × 10^4 cells/mL) were cultured in 3D-printed scaffolds for 7 and 14 days. At the indicated time point, RNA was extracted from the cells, and 1 μg of RNA was reverse transcribed into complementary DNA (cDNA). siRNA Transfection Reagent (QIAGEN, Hilden, Germany) was utilized following the manufacturer's protocol. The expression of actin, osteocalcin (OCN), alkaline phosphatase activity (ALP), runt-related transcription factor 2 (Runx), and type I collagen (Col-I) were quantified and analyzed in triplicate. The relative expression level of each gene was measured and the expression level of each target gene was normalized to the reference housekeeping gene (GAPDH).

2.7. In vivo implantation of PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds

Twenty-four Sprague Dawley (SD) rats (weight: 200 ± 20 g) bearing a circular thighbone defect (diameter [mm]: 4) were divided into four groups. 3D-printed PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds with 25% TCP content (diameter [mm] × height [mm]: 4 × 6) were implanted in the thighbone defect of the rats. New bone formation was measured using micro-computed tomography (Skyscan1276 X-Ray Microtomo-graph, Bruker, Belgium). New bone area and percentage were determined.

3. Results and discussion

3.1. Production of PTMC/TCP, PCL/TCP, and PTMC/PCL/TCP scaffolds

Biodegradable PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds were produced using 3D printing technique and further characterized. Chemical characterization of PTMC, PCL, TCP, PTMC/TCP, PCL/TCP, and PTMC/PCL/TCP scaffolds was analyzed using Fourier-transform infrared spectroscopy. The spectra of PTMC/TCP displayed characteristic 1740 cm\(^{-1}\) peaks of ester and 2900 cm\(^{-1}\) peaks of CH, and accordingly, peak intensities decreased with increasing of TCP content from 0% to 25% (Figure 1A). Moreover, PTMC/TCP indicated 1040, 960, and 564 cm\(^{-1}\) peaks of P-O and P=O. PCL/TCP also displayed typical 1740 cm\(^{-1}\) peaks of ester, 2900 cm\(^{-1}\) peaks of CH, and 1040, 960, 603, and 564 cm\(^{-1}\) peaks of spectral features of P-O and P=O (Figure 1B). Meanwhile, the spectra of PTMC/PCL/TCP also demonstrated 1948 cm\(^{-1}\) peaks of typical ester, 2960 cm\(^{-1}\) peaks of CH, and accordingly, peak intensities decreased with increasing of TCP content (Figure 1C). Moreover, PTMC/PCL/TCP scaffolds showed P-O and P=O peaks at 1181, 960, 603, and 564 cm\(^{-1}\).

Figure 2 demonstrates that the water contact angles of all scaffolds increased with TCP content. Moreover, PTMC/PCL/TCP scaffolds showed higher water contact angles than PTMC/TCP scaffolds and lower water contact angles than PCL/TCP scaffolds. This result indicated that PTMC/PCL/TCP scaffolds possessed lower hydrophilicity than PTMC/TCP and higher hydrophilicity than PCL/TCP scaffolds. Therefore, the modification of PCL with PTMC can enhance hydrophilicity of composites.

3.2. Scanning electron microscopy of PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds

Micrographs of all scaffolds were measured by a scanning electron microscope. Figure 3A-C indicates that TCP scattered uniformly and presented a few aggregations in the scaffolds. The intensity of TCP appeared to increase on the basis of the enlargement of TCP content. Therefore, TCP displayed good compatibility to PTMC and PCL. The micrographs of PTMC/PCL/TCP scaffolds indicate that TCP can enhance the compatibility of PCL and PTMC, in a way similar to the thermals properties above. The phase composition was measured by X-ray diffraction diffractometer (XRD, German Bruker Co., Germany). PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds displayed the same typical XRD data as PTMC, PCL, and TCP (Figure 4A-C).

Compressive modulus of scaffolds was determined using MTS universal electronic testing machine according to the standard GB/T 1041-2008; the results are presented in Figure 5A-C and Table 1. All scaffolds showed an increase in compressive modulus with the increase of TCP content. PTMC/PCL/TCP scaffolds displayed higher compressive modulus than PTMC/TCP and lower values.
than PCL/TCP scaffolds. Compressive moduli of human compact bone and cancellous bone were 14 – 20 GPa and 97.8 – 800 MPa, respectively, while compressive modulus of cartilage was 0.4 – 0.8 MPa \cite{32,33}. Therefore, PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds displayed lower compressive modulus than human compact bone and obviously higher compressive modulus than cartilage. Moreover, compressive moduli of PCL/TCP and PTMC/PCL/TCP scaffolds were just within the compressive modulus requirements of cancellous bone. Therefore, PCL/TCP and PTMC/PCL/TCP scaffolds are suitable for use in repairing cancellous bone defects. Meanwhile, TCP promoted the compressive property of scaffolds, and the modification of PCL improved the compressive property of PTMC/PCL/TCP scaffolds.

### 3.3. Cell cytotoxicity assay

PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds enhanced cell growth of MC3T3-E1 cells (Figure 6). All scaffolds displayed no significant differences in cell proliferation on day 1. PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds displayed lower cytotoxicity and higher proliferation after 3 days of cultivation compared to only 1 day. PTMC/PCL/TCP scaffolds indicated slightly higher cell growth activity than PCL/TCP and PTMC/TCP scaffolds. Thus, PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP exhibited low cytotoxicity, increased the proliferation of MC3T3-E1 cells, and promoted cell growth.

Based on the above measurement, PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds with 25% TCP content (PTMC/25%TCP, PCL/25%TCP, and PTMC/PCL/25%TCP) were applied to investigate the degradation, drug-release property, cell proliferation rate, and implantation. Meanwhile, PCL/10%TCP, PTMC/10%TCP, and PTMC/PCL/10%TCP were used as the contrast controlled samples.

### 3.4. Degradation

All scaffolds displayed M\textsubscript{r} loss and weight loss in PBS (Figure 7A and B). PTMC/PCL/TCP scaffolds showed the similar weight loss and M\textsubscript{r} loss pattern as that of PCL/TCP scaffolds after degradation for 6 months. PTMC/25%TCP scaffolds displayed similar low weight...
loss and higher $M_n$ loss than PTMC scaffolds. PTMC/ PCL/25%TCP underwent higher weight loss and lower $M_n$ loss than PTMC/PCL scaffolds, while PTMC/ PCL/25%TCP showed a slight weight loss compared to PTMC/PCL/10%TCP scaffolds. PCL/10%TCP underwent relatively higher $M_n$ loss than PCL/25%TCP and PCL scaffolds. PTMC/PCL/25%TCP had lower $M_n$ loss than PTMC/PCL/10%TCP and PTMC/PCL scaffolds. Thus, TCP nanoparticles probably induced the occurrence of lower weight loss and higher $M_n$ loss, increasing of water permeation in composites, and further acceleration of hydrolysis and degradation.

Figure 3. The micrographs of (A) poly($\varepsilon$-caprolactone) (PCL)/$\beta$-tricalcium phosphate (TCP), (B) poly(trimethyl carbonate) (PTMC)/TCP, and (C) PTMC/ PCL/TCP scaffolds with different TCP content. PTMC/TCP scaffolds with (a$_1$) 0%, (b$_1$) 5%, (c$_1$) 10%, (d$_1$) 15%, (e$_1$) 20%, and (f$_1$) 25% TCP content; PCL/ TCP scaffolds with (a$_2$) 0%, (b$_2$) 5%, (c$_2$) 10%, (d$_2$) 15%, (e$_2$) 20%, and (f$_2$) 25% TCP content; PTMC/PCL/TCP scaffolds with (a$_3$) 0%, (b$_3$) 5%, (c$_3$) 10%, (d$_3$) 15%, (e$_3$) 20%, and (f$_3$) 25% TCP content.
3.5. DOX release property

All scaffolds demonstrated substantial release rates and steady drug controlled release properties during 130 days of measurement (Figure 8). PTMC/TCP scaffolds with different TCP content showed similar DOX release rates. Compared with PTMC/TCP scaffolds, PTMC/PCL/TCP scaffolds displayed faster DOX release rates, presumably due to the large pores of scaffolds and high drug diffusion coefficient. PCL/TCP scaffolds displayed obviously slower release rates because of its low hydrophilicity, slow degradation rate, and low drug diffusion coefficient. Meanwhile, the DOX release rates of scaffolds increased with the increased of TCP content. PTMC/PCL/25%TCP and PTMC/PCL/25%TCP displayed 18.1% and 14.1% of cumulative DOX release, respectively, which were higher than PCL/TCP (3.1%). Thus, TCP improved the hydrophilicity, drug diffusion coefficients, and DOX release rates of scaffolds. These results are consistent with the micrographs structures of PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds in this study.

3.6. In vitro biocompatibility assay

Porous 3D-printed PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds can provide a large surface area, which
Figure 6. Cell cytotoxicity assays of poly(ε-caprolactone) (PCL)/β-tricalcium phosphate (TCP) (A), poly(trimethyl carbonate) (PTMC) (B)/TCP, and PTMC/PCL/TCP (C) scaffolds with different TCP content to osteoblast MC3T3-E1 cells (*P<0.05 vs. control).

Figure 7. Weight loss (A) and $M_n$ loss (B) of poly(ε-caprolactone) (PCL)/β-tricalcium phosphate (TCP), poly(trimethyl carbonate) (PTMC)/TCP, and PTMC.

Figure 8. (A-C) Release profiles of DOX from poly(ε-caprolactone) (PCL)/β-tricalcium phosphate (TCP), poly(trimethyl carbonate) (PTMC)/TCP, and PTMC/PCL/TCP scaffolds PCL/TCP scaffolds.
Figure 9. Osteoblast MC3T3-E1 cell proliferation assays on poly(ε-caprolactone) (PCL)/β-tricalcium phosphate (TCP), poly(trimethyl carbonate) (PTMC)/TCP, and PTMC/PCL/TCP scaffolds with 25% TCP content.

Figure 10. Gene expression assays on rBMSCs in poly(ε-caprolactone) (PCL)/β-tricalcium phosphate (TCP), poly(trimethyl carbonate) (PTMC)/TCP, and PTMC/PCL/TCP scaffolds with 25% TCP content. Abbreviations: PCL/TCP-7, PCL/25%TCP scaffolds for 7 days; PTMC/TCP-7, PTMC/25%TCP scaffolds for 7 days; PTMC/PCL/TCP-7, PTMC/PCL/25%TCP scaffolds for 7 days; and PTMC/PCL/TCP-14, PTMC/PCL/25%TCP scaffolds for 14 days.

is conducive to cell proliferation, differentiation, and metabolism. Figure 9 indicates that 3D-printed PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds displayed the uniform pore size and micro-network structures.
Figure 11. Bone repairing assays in thigh bone defect in rats. (A) Micro-CT images of thigh bone defect in rats implanted with poly(ε-caprolactone) (PCL)/25%β-tricalcium phosphate (TCP), poly(trimethyl carbonate) (PTMC)/25%TCP, and PTMC/PCL/25%TCP. (B) New formed bone parameters after 6 weeks and 12 weeks of surgery.
Through the porosity test, we found that the porosity of 3D-printed PCL/25%TCP, PTMC/25%TCP, and PTMC/PCL/25%TCP scaffolds was 66.43 ± 2.56%, 58.9 ± 2.81%, and 48.0 ± 1.84%, respectively.

MC3T3-E1 cells were evenly distributed in 3D-printed PCL/25%TCP, PTMC/25%TCP, and PTMC/PCL/25%TCP scaffolds, which provided microenvironment for cell adhesion and regeneration. MC3T3-E1 cells scattered uniformly, adhered, grew and proliferated in scaffolds, and afterward, the cells synthesized and secreted matrix proteins. MC3T3-E1 cells appeared to have good attachment states and proliferated in PCL/25%TCP, PTMC/25%TCP, and PTMC/PCL/25%TCP scaffolds. TCP also promoted the expression of osteoblast-related genes, alkaline phosphatase gene, and bone adhesion gene, leading to the differentiation of osteoblasts as well as the mineralization and formation of bone. Moreover, the degraded Ca and P from TCP in the body can enter the living circulatory system, which can promote the formation of new bone. PTMC/PCL/25%TCP scaffolds displayed better biocompatibility and stimulated a greater extent of cell regeneration than PTMC/25%TCP and PCL25%TCP scaffolds.

3.7. Osteogenic gene expression

Metabolic activity of rBMSCs was studied after 1 and 2 days of post-culturing in 3D-printed PCL/25%TCP, PTMC/25%TCP, and PTMC/PCL/25%TCP scaffolds. mRNA expression results are presented in Figure 10. Cell proliferation on all scaffolds at 1 day was higher than that at 2 days. Moreover, PTMC/PCL/25%TCP and PTMC/25%TCP scaffolds demonstrated higher levels of cellular metabolic activity than PCL/25%TCP scaffolds at 1 day and 2 days. Meanwhile, PTMC/PCL/25%TCP scaffolds showed slightly higher metabolic activity than PTMC/25%TCP at 2 days.

The PCL/25%TCP, PTMC/25%TCP, and PTMC/PCL/25%TCP scaffolds promoted rBMSCs growth and improved the gene expression of actin, ALP, COL, RUNX2, and OCN. Moreover, rBMSCs scattered uniformly and induced adhesion and regeneration in the scaffolds (Figure 10). PTMCPCL/25%TCP and PTMC/25%TCP scaffolds evidently enhanced gene expression of actin, ALP, COL, RUNX2, and OCN.

However, PCL/25%TCP scaffolds appeared to have almost no gene expression at 7 days post-culturing. Meanwhile, PTMC/25%TCP scaffolds showed higher gene expression than PTMC/PCL/25%TCP scaffolds at 7 days post-culturing. Moreover, PTMC/PCL/25%TCP scaffolds demonstrated higher level of gene expression at 14 days post-culturing than that at 7 days post-culturing. Therefore, PTMC/PCL/25%TCP and PTMC/25%TCP scaffolds displayed good biocompatibility, high cell proliferation performances, high metabolic activity, and gene expression of actin, ALP, COL, RUNX2, and OCN.

3.8. In vivo bone regeneration

All scaffolds were embedded in thigh bone defects for 12 weeks. New bone tissue data, including bone tissues volume/total tissue volume (BV/TV, %), trabecular space (Tb.Sp, mm), number of trabecula (Tb.N, mm⁻¹), and trabecular thickness (Tb.Th, mm) were evaluated. As shown in Figure 11A, plenty of new bone tissues were found in thigh defects of the rats and gradually penetrated into either PCL/25%TCP, PTMC/25%TCP, or PTMC/PCL/25%TCP scaffolds after 6 and 12 weeks. Moreover, bone tissues continued growing to form a network, which repaired bone defects in pace with biodegradation of scaffolds. Figure 11B indicates the new formed bone parameters after 6 weeks and 12 weeks of surgery.

More formation of new bones appeared in thigh defects of the rats implanted with PTMC/PCL/25%TCP and PCL/25%TCP scaffolds than in those with PTMC/25%TCP scaffolds after 6 weeks and 12 weeks. The formation of new bone in PCL/25%TCP and PTMC/PCL/25%TCP scaffolds was higher than that in PTMC/25%TCP scaffold. Therefore, PCL/25%TCP and PTMC/PCL/25%TCP scaffolds can display high osteogenic activity, suggesting its role in bone repair.

4. Conclusion

3D-printed PCL/TCP, PTMC/TCP, and PTMC/PCL/TCP scaffolds provide good porous growth microenvironments and mechanic supports for MC3T3-E1 cells and rBMSCs, and also enhance the proliferation of osteoblast cells. Moreover, PTMC/PCL/TCP and PCL/TCP scaffolds display good biodegradability, good biocompatibility, and high osteogenic activity, which can be used to repair bone defects in vivo.

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Conflict of interest

All authors declare that they have no conflicts of interest.

Author contributions

G-P.Y. designed the experiments. F.L., S-Y.Z., and Z-W.L. manufactured and characterized the samples. H-L.K. and F.L. carried out the in vitro and in vivo experiments. F.L. and G-P.Y. wrote the paper and conducted the analysis and discussions. All authors discussed the results and commented on the manuscript. S-Y.Z. and Z-W.L. contributed equally to this work.

Ethics approval and consent to participate

All animal experiments were performed at the Tongji Hospital of Huazhong University of Science and Technology in accordance with protocols approved by the Institutional Animal Care and Use Committee.

Consent for publication

Not applicable.

Availability of data

The data that support the findings of this study are available from the corresponding author on reasonable request.

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