Biocompatibility, degradability, bioactivity and osteogenesis of mesoporous/macroporous scaffolds of mesoporous diopside/poly(l-lactide) composite

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Bioactive mesoporous diopside (m-DP) and poly(l-lactide) (PLLA) composite scaffolds with mesoporous/macroporous structure were prepared by the solution-casting and particulate-leaching method. The results demonstrated that the degradability and bioactivity of the mesoporous/macroporous scaffolds were significantly improved by incorporating m-DP into PLLA, and that the improvement was m-DP content-dependent. In addition, the scaffolds containing m-DP showed the ability to neutralize acidic degradation products and prevent the pH from dropping in the solution during the soaking period. Moreover, the scaffolds containing m-DP enhanced attachment, proliferation and alkaline phosphatase activity of MC3T3-E1 cells, which were also m-DP content-dependent. Furthermore, the histological and immunohistochemical analysis results showed that the scaffolds with m-DP significantly promoted new bone formation and improved the materials degraded in vivo, indicating good biocompatibility. The results suggested that the mesoporous/macroporous scaffolds of the m-DP/PLLA composite with osteogenesis had a potential for bone regeneration.

1. Introduction

The diopside (DP) bioceramics have been reported to be degradable and have the ability to induce apatite formation on their surfaces in simulated body fluid (SBF), and supported human osteoblastic-like cell attachment, proliferation and differentiation [1,2]. The silicate ceramics in CaO–SiO2–MgO systems have been reported to produce some ions (such as Ca, Mg and Si) that could stimulate osteoblast proliferation and differentiation [3,4]. Another study has reported that the DP ceramics could be used not only for bone tissue engineering but also for periodontal tissue engineering owing to their excellent in vitro and in vivo osteogeneis/cementogenesis [5,6].

A nanostructured diopside (n-DP) was coated on AZ91 magnesium alloy through combined micro-arc oxidation and electrophoretic deposition methods, and the results showed that the n-DP coating increased the corrosion resistance and improved the in vitro bioactivity of the biodegradable magnesium alloy [7,8]. In addition, the n-DP coating on magnesium implants significantly enhanced cell viability and new bone formation compared with the uncoated magnesium implants [7,8]. Moreover, well-ordered mesoporous diopside (m-DP) with a mesopore size of 4 nm was synthesized by the template technique, and the results revealed that the m-DP with large surface area/high pore volume not only improved hydrophilicity, in vitro bioactivity and degradability of DP, but also possessed good haemostatic properties, and could be applied as a haemostatic agent for surgical haemostasis [9].
Biodegradable polyesters, such as poly(ε-caprolactone) (PLLA), polyglycolic acid and their copolymers, have been widely applied for bone regeneration owing to their degradability, biocompatibility and processability [10–12]. However, there are still some problems that need to be solved for PLLA application in the biomedical field, such as the hydrophobicity, lack of bioactivity and the release of acidic degradation by-products, which might cause inflammation when implanted in vivo [13,14]. It is well known that inorganic bioactive materials, such as hydroxyapatite, tricalcium phosphate, bioglasses and calcium silicate, have been incorporated into degradable polymers to develop inorganic/organic biocomposites [15,16]. The combination of bioactive materials with degradable polymers would lead to polymer-based composites with improved physico-chemical and biological properties when compared with polymers alone [17].

To the best of our knowledge, no previous studies have reported the preparation of m-DP/PLLA composite scaffolds with a mesoporous/macroporous structure for bone regeneration applications. We expected that m-DP with high specific surface area/pore volume incorporated into PLLA would improve the physico-chemical and biological properties of the composite scaffolds. Therefore, in this study, the m-DP/PLLA composite scaffolds were fabricated, and the in vitro degradability, bioactivity, cellular responses and in vivo osteogenesis of the scaffolds were investigated.

2. Material and methods

2.1. Preparation of mesoporous diopside and scaffolds

A total of 4.0 g of P123 (EO20PO70EO20, 5800) was dissolved in hydrochloric acid solution (120 mL), which was stirred at room temperature until clear. Then, 4.8 g magnesium nitrate hexahydrate and 5.2 g calcium nitrate tetrahydrate were put into the solution, followed by the dropwise addition of 22.2 g of tetraethyl orthosilicate and magnetic stirring at 50 °C for 5 h and at 80 °C for the next 24 h. The acquired white suspension was washed thoroughly with deionized water, and then dried at 80 °C under vacuum to obtain the powders. Finally, the powders were calcined at 600 °C for 6 h at a heating rate of 1 °C min⁻¹ to remove the template, and the m-DP was obtained. The morphology and microstructure of m-DP were observed using transmission electron microscopy (TEM; JEM2010; JEOL, Japan) and scanning electron microscopy (SEM; S-3400N; Hitachi, Japan).

The PLLA (4032D) was purchased from Nature Works LLC (USA), and the scaffolds of PLLA and m-DP/PLLA composites with 20 wt% (C20) and 40 wt% m-DP (C40) content were prepared by the solution-casting and particulate-leaching method. Briefly, PLLA particles were dissolved in dichloromethane, and m-DP powders were added into the PLLA solution. Afterwards, sodium chloride particles, sieved with diameter of 400–500 μm, were mixed into the compound after uniform stirring. The samples were consolidated in a stainless steel mould (mixed into the compound after uniform stirring. The samples were washed thoroughly with deionized water, then dried at 80 °C in an oven for 12 h. The degradability of samples was determined by testing the weight loss according to the following equation:

$$\text{weight loss(\%) } = \frac{(W_0 - W_t)}{(W_0)} \times 100,$$

where $W_0$ is the starting dried weight and $W_t$ is the dried weight at time $t$.

The pH value of the Tris–HCl solution containing the scaffolds was measured by pH meter (FE20 K; Mettler Toledo, Switzerland) at 7, 14, 21, 28, 35, 49, 63 and 84 days.

2.3. In vitro bioactivity of scaffolds in simulated body fluid

The in vitro bioactivity of the scaffolds (Φ12 × 2 mm) was determined by testing the apatite formation on the specimen surfaces after the samples had been immersed in SBF (pH = 7.40) at 37 °C for 7 days. The surface morphology of the scaffolds was observed by SEM, and the surface composition was determined by energy dispersive spectroscopy (EDS; Falcon, USA) and X-ray diffraction (XRD; Rigaku Co., Japan) after the samples had been soaked in SBF for 7 days. The ion concentrations (Si, Ca, Mg ions) in the SBF were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; IRIS 1000; Thermo Elemental, USA) after the scaffolds soaking into SBF at 1, 3, 5, 7 and 10 days.

2.4. Cell culture on scaffolds

All the scaffolds of PLLA, C20 and C40 (Φ12 × 2 mm) were sonicated in ethanol and sterilized in an autoclave at 120 °C for 30 min. The osteoblast-like cell line MC3T3-E1 (ATCC; Chinese Academy of Sciences, Shanghai, China) was cultured in a 37.4°C (100 r.p.m.) for 84 days, and the Tris–HCl solution was refreshed once a week. At specific time intervals (1, 3, 5, 7, 14, 21, 28, 35, 49, 63 and 84 days), the specimens were washed thoroughly with deionized water, then dried at 80 °C in an oven for 12 h. The degradability of samples was determined by testing the weight loss according to the following equation:

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2.4.1. Cell morphology

The MC3T3-E1 cells were seeded on the scaffolds (Φ12 × 2 mm) in a 24-well plate at 37.5 °C and under a 100% humidity atmosphere with 5% CO₂. The cell seeding density was 1 × 10⁵ cm⁻². After the cells had been cultured for 1, 3 and 7 days, the specimens were washed gently three times with phosphate-buffered saline (PBS) to remove the unattached cells. Then, the cells that were adhered to the samples were fixed in 4% paraformaldehyde for 15 min. After washing with PBS for three times, the cells on the scaffolds were stained with fluorescein isothiocyanate (FITC; Sigma) and 4,6-diamidino-2-phenylindole (DAPI; Sigma). The cell morphologies on the scaffolds at different time were visualized using confocal laser scanning microscopy (CLSM; Hitachi).

2.4.2. Cell proliferation

Cell proliferation was investigated by the CCK-8 assay. The MC3T3-E1 cells (1 × 10⁴ cm⁻²) were seeded on the scaffolds (Φ12 × 2 mm) in 24-well plates, and cells were incubated for 1, 3 and 5 days under a humidified atmosphere at 37 °C and 5% CO₂. The solution was refreshed every 2 days. At each time point, a total of 450 μL DMEM and 50 μL of CCK-8 solution
(Dojindo Molecular Technologies Inc., Kumamoto, Japan) was added to each well and incubated for 3 h. After this incubation, 100 μl of the supernatant was transferred into a 96-well plate and read at 450 nm using a microplate reader (Synergy HT; Biotek, Winooski, VT) with 620 nm as the reference wavelength. The mean absorbance value/optical density (OD) obtained from the blank control was subtracted from the ODs of the test groups.

2.4.3. Alkaline phosphatase activity

The alkaline phosphatase (ALP) activity of cell lysates at 7, 10 and 14 days of growth was measured with the ALP assay in osteogenic medium. Briefly, 1 × 10^5 of MC3T3-E1 cells were seeded on the sample (PLLA, C20 and C40, 8 μl × 2 mm) in 24-well plates and then cultured at 37°C and 100% humidity atmosphere with 5% CO2. At a specific time point, the cell lysate was obtained by adding 1 ml of 0.2% Nonidet P-40 solution (NP-40) to each well at room temperature for 1 h. Then, 50 μl of 1 mg ml⁻¹ p-nitrophenylphosphate (Sigma, USA) substrate solution (pH 9), which contained 0.1 mol l⁻¹ glycine and 0.5 mmol l⁻¹ MgCl₂ in 1 M diethanolamine buffer, was added to each well and incubated at 37°C for 15 min. The reaction was stopped by 100 μl of 0.1 M sodium hydroxide. Then, the OD value was quantified with a microplate reader (SPECTRA Amax 384, Molecular Devices, USA) at a wavelength of 450 nm. The ALP activity was expressed as the OD value per total protein. The total protein content was determined using the BCA protein assay kits and a series of bovine serum albumin standards. ALP activity of cell lysates was normalized to the total protein content of each lysate.

2.5. Animal experiments

This animal model study was approved by the Research Center for Laboratory Animals of the Shanghai University of Traditional Chinese Medicine. Twenty-four healthy New Zealand white rabbits (male, about 2.0 kg) were chosen for surgery. The rabbits were anaesthetized with pentobarbital sodium. The incision sites were made on the left leg of the rabbits, and then 6 mm radial defects were introduced in the thighbone of the rabbits. The scaffolds (8 × 6 × 6 mm) were placed into 4% neutral-buffered formalin. After 2.5.2. Histological and immunohistochemical evaluation

After μCT scanning, samples (8 × 6 × 6 mm) were decalcified in 10% ethylene diamine tetraacetic acid (pH 7.0) for about 30 days at room temperature, then the samples washed with double-distilled water gently, dehydrated in ascending grades of alcohol and embedded in paraffin. A microtome was used to obtain 20 μm thick serial sections of all samples, which were then frozen at −20°C. All sections were stained with haematoxylin-eosin (H&E) or Masson’s trichrome. The newly formed bone was observed under a light microscope (TE2000U, Nikon, Japan). The ratios of mature bone and residual material was calculated according to the following equation:

\[
\text{mature bone ratio} \left( \% \right) = \frac{\text{mature bone area} \ (\text{cm}^2)}{\text{all area} - \text{blank} \ (\text{cm}^2)} \times 100\% \]

and

\[
\text{residual material ratio} \left( \% \right) = \frac{\text{residual material area} \ (\text{cm}^2)}{\text{all area} - \text{blank} \ (\text{cm}^2)} \times 100\%.
\]

2.6. Statistical analysis

All quantitative data expressed as the mean ± s.d. were analysed with Origin v. 8.0 (OriginLab Corporation, USA). Statistical comparisons were carried out using analyses of variance. Statistical significance was attained with a greater than 95% confidence level (p < 0.05).

3. Results

3.1. Morphology of mesoporous diopside and scaffolds

Figure 1a presents TEM images of the morphology of m-DP. It can be seen that the m-DP had highly ordered mesopores with a pore size of about 5 nm [18]. Figure 1b shows SEM images of the morphology of m-DP. The m-DP consisted of rod-like particles with a size of around 5 μm.

Figure 2 presents the SEM images of the surface morphology and microstructure of the fabricated PLLA, C20 and C40 scaffolds under different magnifications. It can be seen that all the scaffolds exhibited macroporous structure with a macropore size of around 400 μm. The results show the m-DP/PLLA composite scaffolds with mesoporous/macroporous structure that were prepared in this study.

3.2. Scaffold degradation in Tris–HCl solution

Figure 3a shows weight loss of the PLLA, C20 and C40 scaffolds after they were soaked in Tris–HCl solution for different times. It was found that weight loss of all the samples increased with time, and the weight loss of the C40 scaffolds was 39 wt% after immersion in Tris–HCl solution for 84 days, which was significantly higher than C20, which had a weight loss of 17 wt%, and PLLA scaffolds, which had a weight loss of 8 wt%. The results indicated...
that the degradability of the scaffolds increased with the rise of m-DP content.

Figure 3b shows the change in pH value of the solution with time after the PLLA, C20 and C40 scaffolds had been soaked in the Tris–HCl solution. For PLLA, the pH of the solution decreased from the initial value of 7.40–6.15 during the whole soaking time. For C20 scaffolds, the pH decreased from 7.40 to 6.80. However, for C40 scaffolds, the pH increased from 7.40 to 7.85.

### 3.3. Apatite formation on scaffolds in simulated body fluid

Figure 4 shows the SEM images of the surface morphology of PLLA (a,b), C20 (c,d) and C40 (e,f) scaffolds at different magnifications. Nevertheless, the surfaces of both the C20 and C40 scaffolds were covered by spherical-shaped apatite. In addition, there was obviously much more apatite on the C40 scaffolds than there was on the C20 scaffolds. The results indicate that incorporation of m-DP into PLLA could enhance the apatite formation (bioactivity) ability of the scaffolds, which was dependent on the m-DP content.

Figure 5 shows the EDS of the surfaces of the C40 scaffolds before and after being soaked in SBF for 7 days. The results indicate that P peaks appeared in figure 5b, which did not exist in figure 5a, and the Ca peaks became higher after the scaffolds were soaked in SBF, indicating that apatite could form on the C40 scaffolds. The EDS results were consistent with SEM.
Figure 6 presents the XRD of the C40 scaffolds before (a) and after (b) being soaked in SBF for 7 days. The diffraction peaks at approximately $2\theta = 25.9^\circ$, $31.8^\circ$, $40^\circ$, and $46.7^\circ$ are attributed to the characteristic peaks of apatite on the surface of the scaffolds.

Figure 7 shows the changes in concentration of P, Ca, Si and Mg ions in solution after the C40 scaffolds were soaked in SBF for different periods of time. The Ca and Mg ion concentration increased around the first to second day, and then decreased up to 10 days. In addition, the Si ion concentration increased at a slow rate, whereas the P ion concentration gradually decreased during the whole soaking period.

3.4. In vitro cytocompatibility

3.4.1. Cell morphology of scaffolds

Figure 8 shows the CLSM images of the cytoskeletons stained by FITC and DAPI after MC3T3-E1 cells were cultured on PLLA, C20 and C40 scaffolds for different numbers of days. It is obvious that the number of cells on the scaffolds increased with time, indicating good cytocompatibility of the scaffolds. Furthermore, it was found that the number of cells on the scaffolds increased with m-DP content.

3.4.2. Cell proliferation on scaffolds

Figure 9 shows the OD values of MC3T3-E1 cells cultured on PLLA, C20 and C40 scaffolds at 1, 3 and 5 days. It can be seen that the OD value for all the samples increased with time, indicating good cytocompatibility of the samples. At day 1, there was no obvious difference in OD value for all samples. However, the OD value for the C40 was significantly higher than C20 and PLLA scaffolds at days 3 and 5. Moreover, the OD value for the C20 was significantly higher than for the PLLA scaffolds at days 3 and 5.

3.4.3. Alkaline phosphatase activity of cells on scaffolds

Figure 10 shows the ALP activity of MC3T3-E1 cells cultivated on PLLA, C20 and C40 scaffolds after 7, 10 and 14 days. It was found that the ALP activity for C40 was significantly higher than that for the C20 and PLLA scaffolds after 10 days. Moreover, the ALP activity for C40 was significantly higher than that for the C20 scaffolds, and the ALP activity for C20 was obviously higher than that for the PLLA scaffolds. The results indicate that the ALP expression levels of the cells on the scaffolds increased with the rise of m-DP content.

3.5. New bone growth into scaffolds in rabbit femoral defects

Figure 11 shows the cross sections of three-dimensional µCT reconstruction images after PLLA (a), C20 (b) and C40 (c) scaffolds were implanted into rabbit femur bone defects for different periods of time. It was found that the new bone gradually grew into all the scaffolds, whereas the scaffolds degraded with time. It is easy to see that the C40 scaffold was the best for bone defect repair.

Figure 12 shows the histological sections of H&E staining of PLLA, C20 and C40 scaffolds 4, 8 and 12 weeks after implantation in vivo. It was found that the amount of new bone increased with increasing time and m-DP content in the
scaffolds. In the meantime, the scaffolds reduced. At 12 weeks, a large quantity of new bone was found, and the mature bone was also found in C40 scaffolds. Figure 12 shows the quantitative analysis of mature bone, new bone and residual material by histological section after the scaffolds had been implanted in vivo for different times. It was found that the quantity of new bone and mature bone significantly increased with time and m-DP content in the scaffolds. During these time periods, the residual material gradually decreased.

Figure 13a shows the Masson trichrome staining of the scaffolds 4, 8 and 12 weeks after implantation in vivo, which provided the supplementary information for the H&E staining. It was found that the amount of new bone also...
increased with time and m-DP content in the scaffolds. Meanwhile, the scaffolds gradually reduced. Figure 13b shows the quantitative analysis of mature bone, new bone and residual material by histological section (Masson trichrome staining) for different time periods after the scaffolds were implanted in vivo. It was found that the quantity of new bone and mature bone significantly increased with time and m-DP content while the residual material decreased. The Masson trichrome staining results were in accordance with H&E staining.

3.6. Immunohistochemistry of new bone formation

Figure 14a shows the images of histological sections of immunohistochemical staining of BMP-2 for 4, 8 and 12 weeks after the PLLA, C20 and C40 scaffolds were implanted in vivo. The figure shows that the BMP-2 staining intensity (brown) for all the scaffolds increased with implant time and m-DP content in the scaffolds. At 12 weeks, the BMP-2 staining intensity for C40 was obviously higher than that for the C20 and PLLA scaffolds.

Figure 14b shows the positive expression ratio of BMP-2 for PLLA, C20 and C40 scaffolds for different periods of time. It was found that the positive expression ratio for all the scaffolds increased with time and m-DP content in the scaffolds. The positive expression ratio of BMP-2 for the C40 scaffolds was 57%, which was higher than that for the C20 (39%) and PLLA scaffolds (18%) 12 weeks after implantation in vivo.

4. Discussion

In this study, m-DP, one of the magnesium-based biomaterials in the CaO–SiO₂–MgO system, was synthesized and its composite scaffolds with PLLA were prepared by the solution-casting/particulate-leaching method. Our findings showed that the addition of m-DP into PLLA enhanced the degradability and bioactivity of the m-DP/PLLA composite scaffolds, and the composite scaffolds could improve the cell proliferation and ALP activity of MC3T3-E1 cells, which were m-DP content-dependent. The composite scaffolds containing...
m-DP could neutralize the acidic degradation by-products released from the PLLA. Furthermore, the composite scaffolds with mesoporous/macroporous structure (macropore size of around 400 \( \mu \text{m} \)) obviously enhanced the new bone formation and promoted material degradation in vivo.

For bone regeneration applications, bioactive materials with different degradation rates are needed [19]. Moreover, the degradation rate of bone implants should synchronize with the growth of new bone tissue, and the implants could gradually be replaced by new bone tissue in vivo [20]. In this study, it was found that the weight loss of C40 scaffolds was 37 wt% after immersion in Tris–HCl for 84 days, which was higher than the weight loss of C20 scaffolds (17 wt%) and PLLA scaffolds (8 wt%), suggesting that C40 degraded faster than C20 and PLLA scaffolds. Obviously, addition of m-DP into PLLA improved the degradability of the scaffolds, and the degradation rate of the scaffolds increased with the rise in m-DP content. The m-DP with large surface area/high pore volume could enable more liquid to enter into the scaffolds, which might be responsible for enhancing the degradability of the scaffolds.

Some studies have reported that the acidic degradation by-products, released by the PLLA, would decrease the pH value, which might lead to inflammatory responses when implanted in vivo [21,22]. In this study, it was found that there was a gradually decrease in pH of the solution containing the PLLA, indicating that some acidic by-products were produced during the soaking. Furthermore, the pH of the solution for C20 scaffolds showed little decrease (from 7.40 to 6.80). However, the pH of the solution for C40 scaffolds slightly increased from the initial value of 7.40 to 7.85. The results suggest that composite scaffolds containing m-DP could improve the pH value of the solution. A possible explanation for this might be that the alkaline ions (\( \text{Ca}^{2+}, \text{Mg}^{2+} \)) from the m-DP in the scaffolds might neutralize the acidic degradation by-products of PLLA and thus compensate for the decrease in pH value. Therefore, the possible inflammation effects from the acidic degradation products of

![Figure 13](http://rsif.royalsocietypublishing.org/doi/figure/10.1098/rsif.2015.0507)
PLLA could be solved by development of m-DP/PLLA composite scaffolds for bone-regeneration applications. The ability to induce apatite in SBF, as well as the amount of apatite, determines the bioactivity of a biomaterial, and predicts the *in vivo* bone bioactivity. The bioactivity of the biomaterial is an essential role in the formation and maintenance of the tissue–biomaterial interface [23,24]. Previous studies found that DP bioceramics possessed apatite-forming ability in SBF and could closely bond to the bone tissue when implanted *in vivo* [1,2]. In this study, the C20 and C40 scaffolds could induce apatite formation on their surface, whereas no apatite was found on the PLLA scaffolds after soaking in SBF for 7 days, indicating that the composite scaffolds containing m-DP significantly improved the ability to form apatite, which was dependent on the m-DP content. The results indicate that the m-DP content might be one of the key factors which affected the dissolution of the m-DP in the scaffolds, following the nucleation and growth of apatite on the surfaces of the scaffolds in SBF.

Cell responses to a biomaterial, such as proliferation and differentiation, depend not only on surface morphology but also on the chemical composition of the biomaterials [25]. Previous studies have shown that DP bioceramics significantly stimulated the proliferation and osteogenic differentiation of several kinds of stem cells [1,2]. In this study, it was found that the MC3T3-E1 cells could proliferate on the PLLA, C20 and C40 scaffolds, suggesting positive cellular responses to these materials, and indicating good cytocompatibility. Furthermore, the proliferation of cells on the scaffolds was obviously enhanced by the increase in m-DP content (C40 > C20 > PLLA), suggesting that incorporation of m-DP into the scaffolds could significantly improve cell proliferation. The composite scaffolds containing m-DP with huge specific surface area/high pore volume might provide a good opportunity for the interaction between the cells and substrates. Moreover, the surface chemical compositions play a crucial role in determining the cell responses by releasing Ca, Si and Mg ions from the composite scaffolds, facilitating cell proliferation.

ALP activity has been used as an early marker for functionality and differentiation of osteoblasts during *in vitro* experiments, and ALP activity of osteoblasts on the samples could reflect the influence of the material on the cells’ ability.

![Figure 14](http://rsif.royalsocietypublishing.org/)
to form new bone in vivo [26]. In this study, the results showed that the ALP activity of the MC3T3-E1 cells cultured on the scaffolds increased with time and m-DP content, indicating that the scaffolds with high m-DP content obviously improved the ALP activity of the cells. The results suggest that the m-DP content might be one of the key factors which promoted cell differentiation. Studies have shown that the ion-dissolution products containing Ca, Si and Mg from bioactive materials such as bioglass/ceramics could stimulate osteoblast proliferation, differentiation and gene expression [27–29]. In this study, Ca, Si and P ions were continuously released (dissolution) from the composite scaffolds (C40) into the solution during soaking in SBF, which produced a Ca-, Mg- and Si-rich environment that might be responsible for stimulating osteogenic differentiation of MC3T3 cells.

In order to investigate the in vivo osteogenesis and degradation of the scaffolds, the scaffolds were implanted into bone defects in rabbits. The results from histological and immunohistochemical analysis showed that the scaffolds with m-DP significantly promoted new bone formation in vivo, which was m-DP content-dependent. The degradation of biomaterials in vivo has been addressed in many studies, and chemical dissolution and cell-mediated resorption have been suggested [30]. In this study, it was found that the new bone tissue gradually grew into the macro/mesoporous scaffolds with time, accompanied by the degradation of the scaffolds. It might be that the chemical dissolution of a scaffold at an initial stage enlarged its surface area, which might facilitate cell-mediated resorption later. Moreover, the new bone area gradually increased, whereas the scaffolds continued to reduce, revealing that a cell-mediated resorption of composite scaffolds occurred. It is suggested that the macro/mesoporous scaffolds with special structure could promote the degradation of the scaffolds and osteogenesis.

Compared with PLLA scaffolds, it is likely that composite scaffolds containing m-DP with a large surface area/high pore volume might provide a suitable microenvironment for the interaction between cell/tissue and scaffolds, and thus enhance bone tissue growth into the scaffolds in vivo. In addition, the release of Si, Mg and Ca ions from the dissolution of the composite scaffolds produced a microenvironment which might be responsible for stimulating cell/new bone tissue ingrowth into the composite scaffolds. Moreover, m-DP with a large surface area/high pore volume in the scaffolds might enhance degradation of the scaffolds in vivo that promotes bone tissue regeneration. In conclusion, the m-DP/PLLA composite scaffolds with good biocompatibility might have special biofunctions to stimulate bone tissue regeneration and repair bone defects.

5. Conclusion

The m-DP/PLLA composite scaffolds with macro/mesoporous structure were prepared. The results showed that the addition of m-DP into PLLA enhanced the in vitro degradability and bioactivity of the composite scaffolds, which were m-DP content-dependent. Moreover, the scaffolds containing m-DP showed the ability to neutralize the acidic degradation by-products released from PLLA and could prevent the pH value of the solution from decreasing during the soaking period. In the cell culture experiments, the composite scaffolds were found to promote cell proliferation and enhanced the ALP activity of MC3T3-E1 cells, which were also m-DP content-dependent. In animal experiments, histological and immunohistological elevation results revealed that the m-DP/PLLA composite scaffolds with macro/mesoporous structure obviously enhanced new bone formation and promoted material degradation in vivo, indicating good biocompatibility. The results demonstrated that the incorporation of m-DP into PLLA was a useful approach to obtain bicomposite scaffolds with improved properties, which might be applied as biomedical materials for bone regeneration.

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