Preparation and osteogenic properties of magnesium calcium phosphate biocement scaffolds for bone regeneration

To cite this article: X Li et al 2013 JINST 8 C07010

View the article online for updates and enhancements.

Related content

- Experimental investigation of mouse kidney aging with SR PCI technology
  P Yifeng, Z Zehua, D Guohao et al.

- Analysis of femur head microstructure in ovariectomized rats
  C B V Andrade, L P Nogueira, C Salata et al.

- Application of X-ray phase contrast microtomography to the identification of traditional Chinese medicines
  L L Ye, Y L Xue, L H Ni et al.
Preparation and osteogenic properties of magnesium calcium phosphate biocement scaffolds for bone regeneration

X. Li, a Y. Niu, b H. Guo, c H. Chen, a F. Li, d J. Zhang, a W. Chen, d Z. Wu, a Y. Deng, a J. Wei a,1 and C. Liu a

Key Laboratory for Ultrafine Materials of Ministry of Education, East China University of Science and Technology, Shanghai 200237, P. R. China

Department of Orthopaedics, Changhai Hospital, The Second Military Medical University, Shanghai 200433, P. R. China

Shanghai Synchrotron Radiation Facility, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, P. R. China

Department of Pharmaceutics, Shanghai 8th People’s Hospital, Shanghai 200235, P. R. China

E-mail: jiewei7860@sina.com

ABSTRACT: The regenerative treatment of large osseous defects remains a formidable challenge in today. In the present study, we have synthesized biodegradable magnesium calcium phosphate biocement (MCPB) scaffolds with interconnected macroporous structure (100–600 µm), as well as good bioactivity, biocompatibility and proper degradatibility. The results revealed that the porosity increased from 52% to 80% of MCPB scaffolds while the compressive strength decreased from 6.1 MPa to 1.2 MPa. We further assessed the effects of scaffolds on the rabbit femur cavity defect model in vivo by using synchrotron radiation X-ray microCT and microCT imaging, indicating that the MCPB scaffolds underwent gradually degradation and promoted the extensive neo-bone formation.

KEYWORDS: Computerized Tomography (CT) and Computed Radiography (CR); Medical-image reconstruction methods and algorithms, computer-aided so; X-ray radiography and digital radiography (DR)

1Corresponding author.
1 Introduction

Porous scaffold for bone implant was used to repair the variety of bone defects caused by surgery, disease and trauma [1, 2]. The porous scaffold in bone tissue repair was not only play a supporting role to maintain the shape of the existing tissue, but also act as the scaffolds for cells boarding, growth, differentiation and proliferation [3]. This required that the scaffolds should have a three-dimensional structure, a high porosity and appropriate pore size, which suitable for cell/tissue growth, and ben in favor of the discharge of nutrient transfer and metabolites [4, 5]. Some studies showed that the aperture size of the scaffold had an important influence on the growth of new bone tissue in the bone defect area [6, 7]. The bone tissue cannot growth into the interior of scaffold when the pore size less than 100 µm, only when the pore size range 100 µm to 600 µm, the pore structure of the scaffolds was good for bone tissue ingrowth [8, 9].

Excellent porous scaffolds for bone tissue engineering not only should have good biocompatibility, without causing inflammation and graft rejection after implanted in vivo, but also should have excellent surface activity which suitable for cell adhesion and proliferation [10, 11]. The scaffolds should have matching degradation rate in tissue formation, thereby the bone defect repair meanwhile the scaffolds gradually degraded in vivo [12, 13]. In the present study, the MCPB porous scaffolds were prepared, and the in vitro degradation was evaluated through the weight loss
experiment. The in vivo degradation and the new bone tissue formation of the scaffolds were studied through animal experiments, meanwhile, biocompatibility, and ossification performance of the porous scaffolds were investigated.

2 Materials and methods

2.1 Preparation of MCPB powders

The solid phase powders of MCPB consists of the alkaline components of magnesium oxide (MgO) and the acidic components of calcium dihydrogen phosphate (Ca\((H_2PO_4)\cdot H_2O\)) in a molar ratio of 2:1. The MgO powders was prepared by heating magnesium carbonate pentahydrate in a furnace at 1500°C for 6 h. The resultant powder was cooled to room temperature, and then ground in a planetary ball mill for 5 min (500 r/min), followed by sieving (200 mesh). The hydration reaction retarder of MCPB was composed of sodium polyphosphate (STPP) and decahydrate of sodium tetraborate (STB) [14]. A certain amount of retarders was added to MCPB, then blended and pulverized with a ball mill, and finally followed by sieving (200 mesh) to standby [15].

2.2 Fabrication of MCPB scaffolds

A particulate-leaching method was taken to fabricate MCPB scaffolds. Firstly, MCPB powders were mixed up with certain proportion sodium chloride particles (NaCl, 250–500 μm) and the hardener (Saturated sodium chloride solution, the liquid-solid ratio was 0.2/1). The prepared MCPB paste/NaCl mixture was placed in stainless steel mold (Φ 12 × 10 mm) and molded under a pressure of 2 MPa for 1 min. Then, the molded samples were placed in a constant temperature oven at 37°C and 100% relative humidity (RH) for 7 days, and immersed in deionized water to leach out the porogen. Followed by, the samples were immersed in dilute hydrochloric acid (0.2 M), and placed in the ultrasonic cleaning machine ultrasound for 15 minutes to ensure that the pore walls were interconnected. Finally, the samples were washed with deionized water and ethanol washing, and drying to obtain the final product-scaffolds.

2.3 Scaffold characterization

The porosity of the scaffolds was measured using a specific gravity bottle based on Archimedes’ Principle [16]. The prepared porous scaffolds were grinded to level with fine sandpaper at both ends, and the compressive strength was measured by universal testing machine (AG-2000A, Shimadzu, Japan) with the applied load speed of 1 mm/min, and each set of data from at least three parallel experiments. The surface microstructure was observed by scanning electron microscopy (SEM, JSM-6360LV, JEOL, Japan).

2.4 Degradation in Tris-HCl solution

The degradation of MCPB scaffolds was determined in Tris-HCl solution (buffer solution, pH = 7.4) at solid/liquid ratio of 0.1 g/20 mL in bottles, which were placed into a shaker under a constant temperature of 37.5°C and the solution was refreshed every 3 days. The specimens were removed, rinsed with distilled water and dried to a constant weight in an oven. All the values reported are averages of three specimens and the percentage of weight loss was expressed as (weight loss/initial weight) × 100% at different time points.
Table 1. Porosity and compressive strength of MCPB scaffolds.

<table>
<thead>
<tr>
<th>NaCl/MCPB (wt/wt)</th>
<th>Porosity (%)</th>
<th>Compressive strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>52 ± 4</td>
<td>6.1 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>62 ± 3</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>2.5</td>
<td>71 ± 4</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>80 ± 3</td>
<td>1.2 ± 0.1</td>
</tr>
</tbody>
</table>

2.5 Implantation of scaffolds in vivo

This study adhered to the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85e23 Rev. 1985) and was approved by National Engineering Research Center of Tissue Engineering (Shanghai Glarun Life Technology Co., Ltd). The MCPB scaffolds (Φ 5 × 5 mm) with a porosity of 71% were sterilized for in vivo implanted experiments. Twenty-four skeletal mature New Zealand white rabbits with an average weight of 2.5 kg were used at the age of 2 months and fasted 24 h before assay. Surgical intervention was performed under general anesthesia and sterile conditions. The defects were created with a medium speed burr (5 mm diameter and 5 mm depth) in the right femur around the knee of each rabbit. After the bone cavities were carefully cleaned with physiological saline and gauze, the cylindrical scaffolds were implanted into the defects in the rabbit femora and the wounds were sutured. Prophylactic antibiotic was given for 3 days in case of postoperative infection. Three rabbits at each time point were sacrificed for analysis at 1, 3 and 6 months after operation.

2.6 Synchrotron radiation X-ray microCT and microCT analysis

The microstructure of scaffolds and bone defects were evaluated at beamline BL13W of SSRF (Shanghai, China) using a monochromatic beam with an energy of 30 keV with the spot 45 mm (H) × 5 mm (V). In the current study, a High Resolution VHR1:1 detector (9 × 9 µm, British Photonic-Science), sample-to-detector distance of 1.5 m. One thousand projections within an angular range of 180° were taken and the exposure time amounted to 10 ms per projection with the rotary table speed of 0.36°/s. Flat-field corrections were collected to filter the background to obtain the X-ray images. 3D structure was reconstructed using Amira 4.1 through the filtered back-projection algorithm. Continuous Micro-CT images were scanned using microCT (µ-CT80, Scanco Medical AG Company, Switzerland) along the long axis of the specimens with a spatial resolution of 36 microns. The degradation of the material implanted in vivo and new bone tissue ingrowth situation were observed after image gaussian filtering.

3 Results and discussions

3.1 Compressive strength and porosity of the scaffold

Table 1 shows the porosity and compressive strength of the three-dimensional porous MCPB scaffolds prepared by molded-template dissolution method. The porosity of the scaffolds can be obtained by adjusting the proportion in mass of the sodium chloride particles with MCPB powder.
Figure 1. SEM images (a) and SR-microCT image (b) of porous MCPB scaffolds.

from 52 w% to 80 w% while compressive strength changed from 6.1 MPa to 1.2 MPa. Obviously, the compressive strength was inversely proportional to the hole-making agent content. Comprehensive consideration of the experimental results, we selected the NaCl/MCPB by a ratio of 2.5 to prepare porous scaffolds.

3.2 Microstructure of MCPB scaffolds

As show in figure 1, the morphology and pore structure of MCPB scaffolds were obtained by SEM (figure 1a) and SR-microCT (figure 1b), respectively. The scaffolds exhibited an open macropores with the sizes of 200–600 µm and the micropores on the wall of about 100 µm. The macropores were mutually connected by small apertures (about 100 µm) on the wall. The connectivity structure is conducive to bone cell/tissue ingrowth within the porous scaffolds, but also beneficial to the transport of nutrients required for the growth of bone cells/tissue and blood vessel formation [17]. Combined with SR-microCT picture, the results indicated that the materials not only had higher surface porosity, its internal porous structure also for loose, and the porous structure of the material was mutually connected. In the present study, the scaffolds possess a high specific surface area and porosity exceeding 70%. This structure can provide adequate space for a large number of cell adhesion/growth and secretion of extracellular matrix, and promote the cells nutrient exchange and waste discharge in the implanted material.

3.3 Degradation performance of scaffolds

The weight loss rate of MCPB scaffold (porosity 71%) immersed in a Tris-HCl solution for 12 weeks was showed in figure 2. The results showed that the porous scaffolds weight decreased with the increase of soaking time, and the weight loss rate of scaffolds reaching about 70% at 12 weeks. The results indicated that the porous scaffolds continued to degrade over the entire incubation period in Tris-HCl buffer solution.

3.4 SR-microCT and microCT analysis

The repair of the bone defect where the MCPB was implanted in vivo can be observed clearly in SR-microCT images. After implanted for 1 month, the implant still showed a relatively deep color
in SR-microCT images because the material had a high density and strong absorption meanwhile only partial degradation on scaffolds had been found.

The interface between porous scaffolds and the bone tissue was no longer obvious, and new bone trabecular had extended into the defect site as network structure after implanted for 3 months.

After implanted for 6 months, the bone defect had been completely repaired, and a good growth of tissue was observed in the defect site, and there was no difference compared the repaired site with the normal of the bone tissue, the result of central virtual slice and SR-microCT image of the MCPB scaffolds implanted into thighbone of rabbits for 6 months were shown in figure 3.

Figure 2. Weight loss of MCPB scaffolds immersed in Tris-HCl.

Figure 3. Central virtual slice (a) and SR-microCT image (b) of the MCPB scaffolds implanted into thighbone of rabbits for 6 months. Circle shows the implanted area.
Figure 4. Micro-CT images of the bone defects of rabbits implanted with MCPB after 6 (a, b, c) months. Circle shows the implanted area.

The microCT consequences can be seen from figure 4, the cortical bone tissue of the defect site has healed utterly and any tissue loss was not observed after MCPB scaffolds implanted for 6 months. Through the longitudinal section and cross-section, we have observed that the scaffolds completely degraded in the body, the bone defect ad been fully restored, new bone trabeculae arranged reticular and occupied the defect site.

Compared with the microCT, the SR-microCT not only has a high resolution, but also can be used to analyse the density changes of materials and bone by the gray value. The obtained results of SR-microCT make it easier and more straightforward to observe the degradation of implant and new bone tissue formation on the defect site. Shanghai synchrotron radiation source provides additional means and tools for the study on the bone defect repair, as well as provides a novel idea for using medical imaging technology to study the scaffolds repair bone tissue defects [18].

3.5 Osteogenic areas analysis

The data obtained by HE staining method were calculated using Image-Pro Plus and the results were shown in figure 5. The results revealed that the new bone tissue volume increased with the implantation time increasing: the new bone tissue had reached about 34% at 1 month after implanted, reached more than 69% at 3 months, and more than 89% at 6 months.

Combined with the SR-microCT and microCT images, the results illustrated that the porous scaffolds had no negative impact on surrounding tissue after implanted in vivo, and the new tissue was rapid growth to repair the bone defect with the porous scaffolds degradation and absorption. Especially, the phenomenon of growth tremendously appeared between 1–3 months. This is because the scaffold material was divided and embedded by bone tissue. The results disclosed that the MCPB porous scaffolds had good bone biocompatibility and degradability.

4 Conclusions

In the present study, the MCPB scaffolds were successfully fabricated by a particulate-leaching method. The results demonstrated that the MCPB scaffolds with the pore size of 100–600 µm showed interpenetrated macropores. The porosity of MCPB scaffolds increased from 52% to 80%
while the compressive strength decreased from 6.1 MPa to 1.2 MPa. In addition, the porous scaffolds with superior compressive strength and degradation in Tris-HCl solution. Animal experimental results showed that the MCPB scaffolds implanted invivo had excellent biocompatibility, biodegradability and ossification performance. SR-microCT imaging can be used to observe the process of porous scaffolds to repair bone defects. The results indicated that the MCPB porous scaffolds might fulfill the basic requirements for bone repair.

Acknowledgments

This study was supported by grants from the National Natural Science Foundation of China (No. 31100680, No. 81000799), the National Natural Science Foundation of China (No. 81172989, No. 81271705) and Nano special program of Science and Technology Development of Shanghai (No. 12nm0500400).

References


