PREPARATION AND CHARACTERIZATION OF
MACROPOROUS MAGNESIUM PHOSPHATE SCAFFOLD FOR
BONE REGENERATION

Fan Wu¹*, Yung Ngothai¹, Changsheng Liu², Jie Wei²,
Brian O’Neill¹ and Richard Musgrove³

¹School of Chemical Engineering, University of Adelaide, SA, 5005, Australia
²The State Key Laboratory of Bioreactor Engineering, and Engineering Research Center for Biomedical Materials of Ministry of Education, East China University of Science and Technology, Shanghai, 200237, P.R.China
³SARDI Innovative Food and Plants, SA Food Centre, SA, 5010, Australia

*Email: fan.wu@adelaide.edu.au

ABSTRACT

Much attention has been focused on the biomedical applications of magnesium phosphate cement (MPC) as bone substitution material. The use of porous scaffolds with characteristics such as high porosity along with macropores and three-dimensional interconnected pore structures is beneficial for repairing bone defects. MPC has been proven to be degradable and biocompatible, and therefore might be applied as three-dimensional scaffolds for bone regeneration. In this study, macroporous magnesium phosphate scaffolds were fabricated by the particulate leaching method using sodium chloride as porogen. The morphology, chemical composition and cellular response to the scaffolds were investigated. The obtained scaffold had a well-interconnected porous structure with pore sizes ranging from 400 to 500 µm. The highest porosity determined using the Archimedes’s Principle could reach 71%. X-ray diffraction pattern revealed the main composition of the scaffold was \( \text{NH}_4\text{MgPO}_4\cdot6\text{H}_2\text{O} \). The result of MTT test demonstrated that the obtained scaffold was cytocompatible and had no negative effects on the proliferation of MG63 cells in vitro. These results suggest that the macroporous MPC scaffolds may have potential applications for bone regeneration.

INTRODUCTION

As for the successful repair of bone defects, the pore structure, morphology, mechanical property and biocompatibility of the porous scaffolds are very important. An ideal scaffold should have suitable porosity and well-interconnected pores to facilitate cell attachment, proliferation and transport of nutrients and metabolic waste (Yunos et al. 2008).

Magnesium phosphate cement (MPC) has been widely used in civil engineering as rapid-repair materials due to the features of fast setting and high early strength. In recent years, clinic applications of MPC as bone substitution materials have attracted much attention (Wu et al. 2008). Liu (2006) first applied MPC as inorganic bone
adhesive in screw fixation, artificial joints fixation and comminuted fracture fixation. The MPC powder consists of a mixture of magnesium oxide (MgO) and ammonium dihydrogen phosphate (NH₄H₂PO₄). The main hydration product is NH₄MgPO₄·6H₂O. Previous research demonstrated that MPC has good in vivo degradability and biocompatibility (Wu et al. 2006; Wu et al. 2009; Yu et al. 2010). Due to these characteristics, MPC is proposed as good scaffold candidate for bone tissue engineering. However, till now, MPC is mainly used in the form of moldable cement paste. The possibility of applying MPC as porous scaffolds has never been studied. In this study, macroporous magnesium phosphate scaffolds were fabricated using the particulate leaching method. The porosity, morphology, mechanical strength, phase composition of the porous scaffolds were investigates. In vitro cell compatibility in terms of cell proliferation of the scaffolds was also studied.

**MATERIALS AND METHODS**

**MPC powder**

The MPC powder was composed of magnesium oxide (MgO) and ammonium dihydrogen phosphate (NH₄H₂PO₄) in a molar ratio of 3.8:1. The MgO was prepared by heating basic magnesium carbonate pentahydrate [(MgCO₃)₄·Mg(OH)₂·5H₂O] in a furnace at 1500°C for 6 hours. The resultant powder was first cooled to room temperature, and then grounded in a planetary ball mill for 5 minutes, followed by sieving through 200 and 300 meshes, respectively. The grains in the range of 200 and 300 meshes were kept for further experiment. All the chemicals used were purchased from Sinopham Chemical Reagent Co., Ltd.

**Preparation of the porous scaffolds**

The macroporous magnesium phosphate scaffolds were prepared via the particulate leaching method using sodium chloride (NaCl) particles as porogen (Wei et al. 2010). The MPC powder was mixed with deionized water at a powder/liquid ratio of 5g/ml using a spatula to form a paste. NaCl particles with sizes in the range of 400-500 µm were added into the cement paste. The mixture of MPC paste and NaCl particles was placed into stainless steel mold (diameter: 6 mm, height: 10 mm) and packed under a pressure of 2 MPa. The samples were stored in a 100% relative humidity environment at 37°C for 2 days, followed by immersion in deionized water for 3 days to leach out of NaCl particles. Afterward, the samples were then dried at 50°C for 6 hours to obtain the porous scaffolds.

**Porosity determination**

Porosity of the scaffolds was determined using the method based on the Archimedes’ Principle (Li et al. 2004). In brief, the porosity (P) was calculated according to the following equation: \( P = \frac{(W_2 - W_3 - W_S)}{(W_1 - W_3)} \times 100\% \). \( W_S \) was the weight of the scaffold sample. The specific gravity bottle filled with ethanol was weighed as \( W_1 \). The scaffold was immersed in ethanol completely to allow the infiltration of ethanol into the pore structure of the scaffold. The weight of the specific gravity bottle containing ethanol and scaffold was recorded as \( W_2 \). The ethanol-infiltrated scaffold
was then removed from the bottle. \( W_3 \) was the weight of the specific gravity bottle with the residual ethanol.

**Mechanical testing**

The surface of the samples was slightly polished before tests. The compressive strength of the as-prepared scaffold was measured on a universal testing machine (AG-2000A, Shimadzu Autograph, Shimadzu Co., Ltd, Japan) at a loading rate of 1 mm/min. Three samples were tested for each group and the results were expressed as mean ± standard deviation (mean ± SD).

**Phase composition and structure characterisation**

The phase composition of the scaffold was characterized by X-ray diffraction (XRD; Rigaku Co., Japan) with Cu K\( \alpha \) radiation and Ni filter (\( \lambda =1.5406\) \( \text{Å} \), 100mA, 40kV) in a continuous scan mode. The \( 2\theta \) range was from 10° to 80° at a scanning speed of 10°/min. The fracture surface morphology of the scaffold was observed with scanning electron microscopy (SEM; JSM6360, JEOL, Japan).

**Cell proliferation**

The proliferation of MG63 osteoblast-like cells cultured on the scaffold samples was assessed quantitatively using methyl thiazoly tetrazolium (MTT) assay. Prior to cell seeding, the scaffold samples (\( \Phi 10 \times 3 \) mm\(^3 \)) were sterilized by autoclaving at 120°C for 20 minutes. The tissue culture polystyrene (TCP) was taken as the control. The scaffold samples were placed in a 24-well plate and the MG63 cells were seeded at a density of \( 5 \times 10^4 \) cells/well. The cell-seeded scaffolds were incubated at 37°C and 100% humidity with 5% \( \text{CO}_2 \) in a DMEM-BFS medium. The culture medium was replaced every two days. After culturing for 3 and 5 days, 100 \( \mu \)L MTT solution was added into each well of the plate. The plate was then incubated for further 4 hours. The supernatant of each well was then removed and 200 \( \mu \)l dimethyl sulfoxide (DMSO) added. After shaking for 10 minutes, the optical density (OD) at 490 nm was measured with an enzyme-linked immunosorbent assay plate reader. Six samples were tested for each culture time and each test was performed in triplicate. A one-way analysis of variance (ANOVA) test was performed to detect significant effects. A \( p \) value < 0.05 was considered to be statistically significant.

**RESULTS AND DISCUSSIONS**

In this study, we prepared macroporous magnesium phosphate scaffolds using the particulate leaching method. So far, several methods have been utilized to fabricate porous scaffolds, such as gas foaming, phase separation, emulsion freeze-drying, three-dimensional printing, etc (Yunos et al. 2008). The particulate leaching method was proved to provide easy control of the pore structure (Chen et al. 2001). Fig.1 presents the macroscopic graph of the as-prepared magnesium phosphate scaffold. The scaffold showed an obvious macroporous structure. The formation of the macropores was led by the leaching of the NaCl particles.
The variation of porosity with the particulate/MPC powder ratio was shown in Fig. 2. The porosity of the scaffold obviously increased with the increasing particulate/MPC powder ratio. The result was in accordance with the previous research that the porogen content had significant influence on the scaffold porosity (Wang et al. 2007). Therefore, the magnesium phosphate scaffold porosity could be adjusted by the control of particulate/cement powder ratio.

Fig. 2 The effect of particulate/MPC powder ratio on porosity of porous magnesium phosphate scaffold

Fig. 3 presented the variation of compressive strength with porosity of the as-prepared magnesium phosphate scaffold. The compressive strength of the scaffolds decreased with the increasing porosity, ranging from 0.8 to 2.2 MPa. The compressive strength of the magnesium phosphate scaffolds was close to the strength of spongy bone (0.2-4 MPa) (Callcut & Knowles, 2002).
Fig. 3 Relationship between porosity and compressive strength of porous magnesium phosphate scaffold

Fig. 4 XRD pattern of the porous magnesium phosphate scaffold

Fig. 4 showed the X-ray diffraction pattern of the porous magnesium phosphate scaffold. The peaks at $2\theta=15.7^\circ$, $16.4^\circ$, $20.8^\circ$, $21.4^\circ$, $27.0^\circ$, $30.6^\circ$, $31.9^\circ$, $33.2^\circ$ and $33.6^\circ$ could be attributed to magnesium ammonium phosphate hexahydrate.
(NH$_4$MgPO$_4$·6H$_2$O), which is the main hydration product of magnesium phosphate cement. The presence of some unreacted MgO particles was confirmed by the peaks at 2θ=42.8° and 62.2°. Thus, the chemical composition of the magnesium phosphate scaffold was a mixture of NH$_4$MgPO$_4$·6H$_2$O and MgO.

In Fig.5, the scaffold exhibited a macroporous structure with pore size mainly in the range of 400-500 µm. SEM micrograph at higher magnification showed that several tiny pores appeared on the wall of the macropores, indicating the formation of an interconnected pore network. It has been found that the macroporous configuration of the scaffold was favorable for tissue ingrowth, cell seeding, cell migration, matrix deposition, vascularization, nutrients transport (Heo et al. 2009). The macropores could help increase the surface area of the scaffolds in contact with the body fluids, accelerating the bioresorption of the scaffolds and facilitating the ingrowth of the new bone tissue (Wei et al. 2010).

**Fig.5 SEM micrographs of the fracture surface of the magnesium phosphate scaffold at magnification of: (a)×25, (b)×100**

**Fig.6** Proliferation of MG63 cells cultured on magnesium phosphate scaffolds for 3 and 5 days (*: statistically different from the control).
In vitro cell culture experiments were performed to evaluate the cytocompatibility of the magnesium phosphate scaffolds. The OD value can provide an indication of cell proliferation on the different materials. The OD value of MG63 cells on both magnesium phosphate scaffolds and the TCP control are shown in Fig.6. The OD value for magnesium phosphate scaffolds significantly increased with the culture time \((p<0.05)\), indicating that the magnesium phosphate scaffolds have no negative effect on the proliferation of MG63 cells. In addition, the OD value for magnesium phosphate scaffolds is significantly higher than that for the control after 3 and 5 days \((p<0.05)\). It indicates that the proliferation of MG63 cells on magnesium phosphate scaffolds is significantly higher than on the control during the culture period. The results suggest that the macroporous magnesium phosphate scaffolds are cytocompatible to MG63 cells and could promote cell proliferation.

CONCLUSIONS

In present study, macroporous magnesium phosphate scaffolds were prepared by the particulate leaching method using sodium chloride as porogen. The so-obtained magnesium phosphate scaffolds possessed a well-developed interconnected porous microstructure. The scaffold porosity could be adjusted by the amount of porogen. The highest porosity could reach 71%. The scaffolds were mainly composed of \(\text{NH}_4\text{MgPO}_4\cdot6\text{H}_2\text{O}\). An in vitro cell-material interaction study, using MG63 cells, indicated that the macroporous magnesium phosphate scaffold was cytocompatible and could promote cell proliferation. The results suggest that the macroporous magnesium phosphate scaffolds developed in present study might be potential candidate for bone regeneration and tissue engineering applications.

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REFERENCES


**BRIEF BIOGRAPHY OF PRESENTER**

Fan Wu is a PhD candidate at School of Chemical Engineering, Adelaide University. She got a Master Degree in Material Science and Engineering from East China University of Science and Technology (ECUST), China. Her research interest is the biomedical materials for bone regeneration, such as bone cement and 3D porous scaffolds.