Original Research

Engineered polycaprolactone–magnesium hybrid biodegradable porous scaffold for bone tissue engineering

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Abstract

In this paper, we describe the fabrication of a new biodegradable porous scaffold composed of polycaprolactone (PCL) and magnesium (Mg) micro-particles. The compressive modulus of PCL porous scaffold was increased to at least 150% by incorporating 29% Mg particles with the porosity of 74% using Micro-CT analysis. Surprisingly, the compressive modulus of this scaffold was further increased to at least 236% when the silane-coupled Mg particles were added. In terms of cell viability, the scaffold modified with Mg particles significantly convinced the attachment and growth of osteoblasts as compared with the pure PCL scaffold. In addition, the hybrid scaffold was able to attract the formation of apatite layer over its surface after 7 days of immersion in normal culture medium, whereas it was not observed on the pure PCL scaffold. This in vitro result indicated the enhanced bioactivity of the modified scaffold. Moreover, enhanced bone forming ability was also observed in the rat model after 3 months of implantation. Though bony in-growth was found in all the implanted scaffolds. High volume of new bone formation could be found in the Mg/PCL hybrid scaffolds when compared to the pure PCL scaffold. Both pure PCL and Mg/PCL hybrid scaffolds were degraded after 3 months. However, no tissue inflammation was observed. In conclusion, these promising results suggested that the incorporation of Mg micro-particles into PCL porous scaffold could significantly enhance its mechanical and biological properties. This modified porous bio-scaffold may potentially apply in the surgical management of large bone defect fixation.

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1. Introduction

Bone tissue engineering offers an alternative solution to the traditional methods of bone replacement including allografts and autografts [1]. Tissue grafting has been used since the 1660s [2]. Bone grafts are the second most common transplanting tissue with more than 2.2 million bone grafting procedures conducted worldwide annually [3,4]. Autografts have been remained as the gold standard in bone transplantation for bridging bone deficiencies such as repairing large bone defects [1,5,6]. Although they possess good osteoinductive and osteoconductive properties, both autografts and allografts have limitations in terms of the availability and donor site morbidity during autologous bone graft harvesting procedures and the risk of disease transmission with the use of allografts [6–8]. Therefore, the use of synthetic scaffold is the most common technique and good approach to regenerate diseased or damaged bone tissue. An ideal bone substitute should possess certain properties including osteoconductivity, biodegradability as well as...
adequate mechanical properties \([9,10]\). Scaffold made of ceramic such as calcium phosphate and calcium sulphate is the most commonly used material for bone regeneration due to its bioactive properties \([11]\). However, its brittleness and fast resorption rate are concerned clinically \([12,13]\). Biodegradable polymer is another type of potential bone graft substitutes. Polycaprolactone (PCL) is one of the suitable candidate, since it is a FDA approved biodegradable polymer with low degradation rate when compared with poly(lactic-co-glycolic acid) (PLGA) and polyactic acid (PLA) \([14]\). However, the low mechanical strength and intrinsic hydrophobic properties of this bio-degradable polymer may limit its use in orthopaedics \([2,15]\). Hence, modification is warranted in order to improve its mechanical and biological properties. Magnesium is a potential additive, as particular amount of magnesium ions may up-regulate the osteogenic markers and promote new bone formation in our previous studies \([16]\). Also, magnesium ion \((\text{Mg}^{2+})\) is essential to human metabolism in which it can affect many cellular functions including the transportation of potassium and calcium ions, the modulation of signal transduction, energy metabolism and cell proliferation \([17,18]\). Furthermore, the literature reported that the majority of magnesium content was found in the bone system \([19,20]\). Therefore, these findings highlighted the significance of magnesium to human body and bone growth. In this study, our group has fabricated a polymeric–metallic hybrid biodegradable porous scaffold made of PCL and magnesium \((\text{Mg})\) micro-particles in order to facilitate bony ingrowth after implantation. This paper reports the mechanical, in vitro and in vivo properties of the newly developed scaffold.

2. Experimental

2.1. Materials

Commercial magnesium particles in micron size \((i.e. \ 45 \mu \text{m and } 150 \mu \text{m})\) (International Laboratory, USA) and polycaprolactone (PCL) (Sigma-Aldrich, USA) with the average molecular weight of Mn \(\sim 80,000 \text{ g/mol}\) were used for the scaffold fabrication. Silane coupling agent, 3-(trimethoxysilyl) propyl methacrylate (TMSPM) (Sigma, USA) was used to coat on the surface of Mg particles in order to enhance the bonding between PCL and Mg. The treatment parameters and the characterisation of the silane coating after treatment were reported in our previous study \([16]\). Salt leaching technique was used for the scaffold fabrication. In brief, 1 g of PCL was dissolved in 10 ml organic solvent, dichloromethane (DCM). After that, 10 ml cold 100% ethanol was added to the PCL polymer solution in order to displace the organic solvent. 0.4 g of Mg micro-particles with either 45 \(\mu \text{m}\) or 150 \(\mu \text{m}\) particle size were then added to the mixture to form the polymer slurry and 7 g of sodium chloride \((\text{NaCl})\) was added and mixed thoroughly. The mixture was pour into a mould and wait overnight until all the solvent evaporated. Finally, the sample was immersed into sodium hydroxide \((\text{NaOH})\) solution to allow the NaCl to leach out in order to obtain porous structure. Four types of Mg/PCL scaffold were fabricated \((i.e. \ 45 \mu \text{m Mg/PCL and } 150 \mu \text{m Mg/PCL scaffolds with and without TMSPM silane coupling agent treatment})\). The volume fractions of Mg particles in the four resultant scaffolds are 20%. Scaffolds with 10 mm \(\times\) 10 mm \(\times\) 5 mm were prepared for both mechanical test and in vitro studies, while scaffolds with 2 mm in diameter and 6 mm in length were prepared for in vivo study.

2.2. Characterisation

The surface morphology of the fabricated scaffolds was examined by scanning electron microscopy (SEM, Hitachi S-3400N) and the porosity of the scaffolds was analysed by using micro-computed tomography (Micro-CT) analysis (SKYSCAN 1076, Skyscan Company). 3D model of the fabricated scaffold was generated by CTVol (Skyscan Company).

2.3. Mechanical test

In order to characterise the effectiveness of the incorporation of Mg particles and also the TMSPM silane coating, compression test was conducted on pure PCL scaffold, uncoupled and silane-coupled Mg/PCL scaffolds. The compression test was performed according to the ASTM D695-08 protocol and the compressive moduli were evaluated after testing.

2.4. In vitro studies

2.4.1. Cell viability of the scaffolds

MTT assay was used to determine the cytotoxicity of the uncoupled and silane-coupled Mg/PCL scaffolds to murine cells. \(7 \times 10^5 \text{ cells/cm}^2\) mouse MC3T3-E1 pre-osteoblasts were cultured in the DMEM culture medium supplemented with 10% \((v/v)\) foetal bovine serum (FBS, Biowest, France), antibiotics (100 U/ml of penicillin and 100 \(\mu \text{g/ml}\) of streptomycin), and 2 \(\mu \text{M}\) \(\text{L-glutamine}\). The cells were seeded on a 96-well tissue culture plate and incubated at 37 °C in an atmosphere of 5% \(\text{CO}_2\) and 95% air for 3 days. After that, 10 \(\mu \text{l}\) of 5 mg/ml MTT solution, which was prepared by dissolving thiazolyl blue tetrazolium bromide powder into the phosphate buffered saline (PBS, OXOID Limited, England) was added to each well and further incubated for 1 day. 100 \(\mu \text{l}\) of 10% sodium dodecyl sulphate (SDS, Sigma, USA) in 0.01 M hydrochloric acid was then added and incubated overnight. Finally, the absorbance was recorded by using multimode detector (Thermo Scientific MULTISKAN GO) at a wavelength of 570 nm with a reference wavelength of 640 nm. The cell viability was then determined from the absorbance.

2.4.2. Cytocompatibility of the silane-coupled Mg/PCL scaffolds

The cytocompatibility of the scaffolds were studied by direct culture using Enhanced Green Fluorescent Protein Osteoblasts (eGFPOB) from GFP mice. The scaffolds were immersed into DMEM culture medium for 7 days prior cell culture in order to enhance the attachment of the cells. \(7 \times 10^5 \text{ cells/cm}^2\) eGFPOB were seeded on each sample in 96-well plate and the cells were cultured in the same condition as in the MTT assay. After 3
days of incubation, the cell morphology was viewed under fluorescent microscopy (Niko ECL IPSE 80i, Japan) equipped with a Sony DKS-ST5 digital camera.

2.5. Bioactivity test

To determine the bioactivity of the scaffolds with and without the incorporation of Mg particles, all the scaffolds were immersed into DMEM culture medium for 7 days. After that, the scaffolds were viewed and examined under scanning electron microscopy (SEM, Hitachi S-3400N) and the surface composition was analysed by energy-dispersive X-ray spectroscopy (EDX).

![Image](image1.png)

**Fig. 1.** Intra-operative picture of scaffold implantation in the lateral epicondyle of SD rat. Black arrow shows the insertion position of the scaffold.

![Image](image2.png)

**Fig. 2.** (a) Surface morphology of the pure PCL and Mg/PCL hybrid porous scaffolds examined by scanning electron microscopy and (b) Micro-CT 3D reconstruction model of the fabricated scaffold.

2.6. In vivo study

2.6.1. Surgical procedure

After characterisation and in vitro studies, animal study was performed to study the biocompatibility of the selected scaffold. The anaesthetic, surgical and post-operative care protocols were examined and fulfilled the requirements of the University Ethics Committee of the University of Hong Kong.

![Image](image3.png)

**Fig. 3.** Compressive moduli of the pure PCL scaffold, Mg/PCL and silane-coupled Mg/PCL porous scaffolds. The compressive moduli of both the Mg/PCL and silane-coupled Mg/PCL scaffolds were found to be significantly higher (*p < 0.05) than the pure PCL scaffold. In addition, the compressive moduli of the TMSPM silane-coupled 150 μm Mg/PCL porous scaffolds was significantly higher (#p < 0.05) than the 150 μm Mg/PCL scaffold.

2-Month old female Sprague–Dawley rats (SD rats) with the average weight of 200–250 g from the Laboratory Animal Unit of the University of Hong Kong were used in this study. Each rat was implanted with pure PCL, or silane-coupled Mg/PCL scaffolds on either the left or right side of the lateral epicondyle. For the surgical operation procedures, the rats were anaesthetized with ketamine (67 mg/kg) and xylazine (6 mg/kg) by intraperitoneal injection. After hair shaving and decortication of the operation site, a hole with 2 mm in diameter and 6 mm in depth was drilled by a hand driller. After that, the pure PCL scaffold and silane-coupled scaffold were implanted into the holes on either left or right femur (Fig. 1) and the wound were then closed layer by layer with proper wound dressing applied. After surgery, 1 mg/kg terramycin (antibiotics) and 0.5 mg/kg ketoprofen (analgesic) were injected into the operated rats through subcutaneous injection. The rats were euthanized 3 months after surgery.

2.6.2. Histological analysis

The bone samples with scaffolds were harvested after 3 months and they were fixed in 10% buffered formalin for 3 days followed by a standard tissue processing procedure. In brief, a dehydrating process was carried out from 70% to 100% ethanol. After that, xylene was used as a transition medium between ethanol and methyl-methacrylate. The samples were immersed in each of the solutions mentioned above for 3 days. Finally, all the bone samples were embedded in methyl-methacrylate (MMA, MERCK, Germany). The embedded samples were cut into sections with 200 μm think and then ground to a thickness of approximately 60 μm. The polished samples were then stained with Giemsa (MERCK, Germany) and the morphological analysis including bone growth and integration with host tissue was examined using optical microscopy.

![Fig. 4. Cell viability of MC3T3-E1 pre-osteoblasts cultured on pure PCL, Mg/PCL and TMSPM silane-coupled porous Mg/PCL scaffolds using MTT assay. Cell viability was found significantly higher in both Mg/PCL and TMSPM silane-coupled porous scaffolds as compared to the pure PCL scaffold (p < 0.05).](image)

![Fig. 5. Microscopic view of (a and d) pure PCL, (b and e) TMSPM-coupled 45 μm particle Mg/PCL scaffold, and (c and f) TMSPM-coupled 150 μm particle Mg/PCL scaffold after cultured with GFP mouse osteoblasts for 3 days. The scaffolds were immersed into the DMEM culture medium for 7 days prior to the cell culturing. 7 × 10^5 cells/cm^2 GFPOB were cultured on each scaffold in the 96-well plate. (a–c) show the scaffolds with lower magnification (20 × ), whereas (d–f) show the scaffolds with higher magnification (40 × ).](image)
3. Results and discussion

3.1. Morphology and porosity of the scaffolds

The surface morphology of the pure PCL scaffold and Mg-incorporated PCL scaffolds is shown in Fig. 2a. The pore size of the scaffolds was in the range from 200 μm to 500 μm. Fig. 2b shows the 3D model of the fabricated scaffold and the porosity of the scaffolds was found to be approximately 74% by Micro-CT analysis.

3.2. The compressive modulus of porous scaffolds

Fig. 3 shows the compressive moduli of the pure PCL, silane-coupled and uncoupled Mg/PCL scaffolds. The compressive modulus of the pure PCL scaffold was found to be 0.4 MPa, while the compressive moduli of the 45 μm and 150 μm Mg micro-particles incorporated scaffolds were 1 MPa and 1.4 MPa, respectively. The compressive property of these modified scaffolds were significantly higher than the pure PCL scaffold. The increased moduli suggested that the addition of the Mg micro-particles was able to enhance the mechanical properties of the PCL scaffolds. Additionally, the compressive moduli were further increased when the Mg micro-particles coupled with TMSPM coupling agent. The increase was significant in particular to the 150 μm incorporated Mg/PCL scaffold. TMSPM is the silane coupling agent commonly used in modifying the adhesion of inorganic particles to polymer matrix [21]. Expectedly, the bonding between PCL and Mg was enhanced after this treatment. Hence, the compressive
modulus of the 150 μm Mg/PCL scaffold was increased to 2.2 MPa, which was doubled as compared with the scaffold without the additional treatment. The silane-coupled 45 μm Mg/PCL scaffold was slightly higher than the uncoupled scaffold; though the difference was found insignificant.

3.3. In vitro cytotoxicity and cytocompatibility of the scaffolds

Fig. 4 shows the cell viability of the pure PCL scaffold and silane-coupled and uncoupled Mg/PCL scaffolds using MTT assay. Significantly higher cell viability was found on all the Mg incorporated scaffolds as compared with the pure PCL scaffold. This result suggested that the release of Mg ions upon degradation was able to enhance the viability of pre-osteoblasts. Previous studies proposed that the presence of magnesium was able to enhance cell adhesion and also stimulated bone growth and bone healing [22–24]. In addition, this in vitro result was consistent to our previous studies, which specific amount of Mg ions released was able to stimulate cellular activity [16]. The presence of Mg micro-particles could improve the overall cellular activity of PCL/Mg scaffold thereof. No significant difference of cell viability was found before and after silane coupling agent treatment. This result hopefully suggested that the silane coating on Mg micro-particles did not jeopardise the cytocompatibility of the hybrid scaffold, while the mechanical property enhanced.

In addition to the cell viability test, the cytocompatibility of all the scaffolds were also examined with the use of the eGFP primary mouse osteoblasts as shown in Fig. 5. Due to the green fluorescent property of the osteoblasts, the green colour represented the living cells. The TMSPM silane-coupled Mg/PCL scaffolds were fully covered by living osteoblasts as compared with the pure PCL scaffold, indicated that the addition of Mg micro-particles was able to enhance cell attachment and growth.

3.4. Bioactivity of the scaffolds

Fig. 6 reveals the surface morphology and composition of the pure PCL scaffold and Mg/PCL scaffolds after 7 days of DMEM immersion. Apart from the detection of Mg element, an apatite layer composed of calcium (Ca) and phosphate (P) was also found on the Mg/PCL scaffolds. However, no apatite layer was found on the pure PCL scaffold, suggested that the Mg/PCL hybrid scaffolds were highly bioactive. Indeed, the literatures mentioned that the apatite layer could provide favourable environment for new bone formation [25,26]. Therefore, the osteoinductivity and osteoconductivity of PCL scaffold could be enhanced after the incorporation of Mg micro-particles [27].

3.5. In vivo study

Fig. 7 displays the histological analysis of bony in-growth and on-growth within and adjacent to the scaffolds implanted for 3 months with the use of Giemsa staining. The purple colour represented the site of new bone formation. The yellow dotted-line circle showed in 40 × magnification photographs indicated the circumference of the scaffolds. After implanted for 3 months, all the scaffolds already degraded and new bone formation was observed within and adjacent to the scaffolds. No sign of inflammation was observed in both pure PCL and Mg/PCL scaffolds. When compared with the pure PCL scaffold, high amount of newly formed bony tissue was
observed in the silane-coupled Mg/PCL hybrid scaffolds. This observation demonstrated that the pores within the scaffolds were interconnected such that bony tissue could grow into the scaffolds. While referring to the histological slides with 200 × magnification, osteoblast-like cells were found next to the newly formed bone (green arrows) in the silane-coupled Mg/PCL hybrid scaffolds. Unfortunately, the mentioned cells did not appear in the pure PCL scaffold. More bone formation found in the hybrid scaffold in vivo was induced by the superior forming ability of apatite layer. All these observations may attribute to the enhancement of scaffold bioactivity due to the release of magnesium ions. Indeed, it was recently reported that the presence of magnesium in the bone system is beneficial to bone growth and also play a key role in bone remodelling and skeletal development [18,20]. Literatures have been shown that the in situ release of magnesium ions is able to stimulate local bone formation and also bone healing by enhancing the osteoblast and osteoclast activities [16,22,23]. However, the amount of Mg ions released is critical for bone formation [28,29]. Too much ions released would lead to bone loss [30]. Hence, in addition to the improvement of mechanical properties of scaffold, the amount of Mg micro-particles incorporated into the scaffold should be carefully controlled so as to avoid any adverse biological effect due to over release of magnesium ions [30,31].

4. Conclusions

This study demonstrates that the incorporation of the magnesium micro-particles to pure PCL scaffold is able to improve its poor mechanical properties and inferior bioactivity. The compressive modulus of PCL scaffold has been significantly increased when magnesium micro-particles added. Its mechanical property can be further enhanced by silane-coupled magnesium micro-particles. Furthermore, the Mg/PCL hybrid porous scaffolds possess superior mechanical property, cytocompatibility and bioactivity as compared with the pure PCL scaffold. All these promising results have shown that the magnesium micro-particle modified porous scaffold is a potential bone substitute candidate for large bone defect fixation.

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