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Original Research

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

Hoi Man Wong[a,c], Paul K. Chu[b], Frankie K.L. Leung[a,c], Kenneth M.C. Cheung[a], Keith D.K. Luk[a], Kelvin W.K. Yeung[a,c,d,*]

[a]*Department of Orthopaedics and Traumatology, The University of Hong Kong, Pokfulam, Hong Kong, China*
[b]*Department of Physics and Materials Science, City University of Hong Kong, Kowloon, Hong Kong, China*
[c]*Shenzhen Key Laboratory for Innovative Technology in Orthopaedic Trauma, The University of Hong Kong Shenzhen Hospital, 1 Haiyuan 1st Road, Futian District, Shenzhen, China*
[d]*Peking University Shenzhen Institution, Gaoxin South 7th Road, Nan Shan District, Shenzhen, China*

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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.


*Keywords:* Magnesium; Polycaprolactone; Biodegradability; Biocompatibility; Mechanical properties

## 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 transplantating 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

*Correspondence to: Department of Orthopaedics and Traumatology, The University of Hong Kong Medical Centre, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong, China. Tel.: +852 2255 4654; fax: +852 2817 4392.
*E-mail address:* wkkyeung@hku.hk (K.W.K. Yeung).

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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 polylactic 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 ($Mg^{2+}$) 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 literatures 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 (Mg) micro-particles in order to facilitate bony in-growth 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 μm and 150 μm) (International Laboratory, USA) and polycaprolactone (PCL) (Sigma-Aldich, USA) with the average molecular weight of Mn ~80,000 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 μm or 150 μm particle size were then added to the mixture to form the polymer slurry and 7 g of sodium chloride (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 (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 μm Mg/PCL and 150 μm 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 × 10 mm × 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. $7 \times 10^5$ 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 μg/ml of streptomycin), and 2 mM L-glutamine. The cells were seeded on a 96-well tissue culture plate and incubated at 37 °C in an atmosphere of 5% $CO_2$ and 95% air for 3 days. After that, 10 μl 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 μl 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 multi-mode 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. $7 \times 10^5$ cells/cm$^2$GFPOB 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