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ENGINEERD MAGNESIUM-BASED RESORBABLE POROUS SCAFFOLD FOR BONE TISSUE ENGINEERING

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INTRODUCTION: Bone tissue engineering offers an alternative solution to the traditional methods of bone replacement including allografts and autografts [1]. Although these biological materials possess good osteoinductive and osteoconductive properties, both of them have limitations in terms of the availability, donor site morbidity and the risk of disease transmission with the use of allografts [2]. 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. 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. However, its brittleness and fast resorption rate are concerned clinically. Polycaprolactone (PCL), a kind of FDA approved biodegradable polymer, is another type of potential bone graft substitutes. However, the low mechanical strength and intrinsic hydrophobic properties of this bio-degradable polymer may limit its use in orthopaedics. 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 [3]. 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.

METHODS: Commercially available magnesium particles in micron size (i.e 45 ?m and 150 ?m) (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 characterization of the silane coating were reported in our previous study [3]. Salt leaching technique was used for the scaffold fabrication in which 7 g of sodium chloride (NaCl) were added and mixed with polymer slurry thoroughly. Finally, the samples were immersed into sodium hydroxide (NaOH) solution to allow the NaCl to leach out in order to obtain porous structure. The surface morphology of the fabricated scaffolds was examined by scanning electron microscopy and the porosity of the scaffolds was analyzed by using micro-computed tomography (Micro-CT) analysis. The compression modulus was examined by Materials Testing System according to the ASTM D695-08 protocol. The samples were cultured with mouse MC3T3-E1 pre-osteoblasts for 3 days

and their cytotoxicity was determined by standard MTT assay. The cell morphology was also examined by using fluorescent microscopy. To determine the bioactivity of the scaffolds, all the samples were immersed into DMEM culture medium for 7 days. Then, the scaffolds were viewed and examined under scanning electron microscopy and the surface composition was analyzed by energy-dispersive X-ray spectroscopy. In the animal testing, the scaffolds were implanted to the lateral epicondyle of femur of 2-month old female Sprague-Dawley rats (SD rats) for 3 months. Bone biopsies were harvested after 3 months. The histological slides were prepared and stained with Giemsa. The bony in-growth and the integration of implant-to-bone were then examined under optical microscopy.

RESULTS: The pore size of all the scaffolds ranged from 200 ?m to 500 ?m and the porosity was found to be approximately 74 %. The compressive modulus of the pure PCL scaffold was found to be 0.4 MPa, whereas the compressive moduli of the 45 ?m and 150 ?m Mg micro-particles incorporated scaffolds increased to 1 MPa and 1.4 MPa, respectively (Fig. 1). Furthermore, the compressive modulus of the 150 ?m Mg/PCL silane coupled scaffold almost doubled to 2.2 MPa as compared with the scaffold without the silane coupling treatment. With respect to the results of in vitro testing, the cell viability was significantly higher in all the Mg incorporated scaffolds as compared with the pure PCL scaffold (Fig. 2). This result suggested that the release of Mg ions upon degradation was able to enhance the viability of pre-osteoblasts. No significant difference of cell viability was found before and after silane coupling treatment. This result hopefully suggested that the silane coating on Mg micro-particles did not jeopardize the cyto-compatibility of the hybrid scaffold, while the mechanical property enhanced. In the bioactivity test, an apatite layer composed of calcium (Ca) and phosphate (P) was found on the Mg/PCL scaffolds, whereas pure PCL porous scaffold did not convince any formation of apatite layer. Referring to the in vivo testing results, all the scaffolds already degraded and new bone formation was observed within the porous structure and also adjacent to the scaffolds after 3-month implantation. No sign of inflammation was observed in both pure PCL and Mg/PCL scaffolds. High amount of newly formed bony tissue was observed in the silane-coupled Mg/PCL hybrid porous scaffolds as compared with the pure PCL porous scaffolds. This observation demonstrated that the pores within the scaffolds were interconnected such that bony tissue could grow into the scaffolds.

DISCUSSION: Previous studies proposed that the presence of magnesium was able to enhance cell adhesion and also stimulated bone growth and bone healing [4]. The current in vitro results were consistent to our previous studies in which specific amount of Mg ions released was able to stimulate cellular activity [3]. The presence of Mg micro-particles could improve the overall cellular activity of PCL/Mg scaffold thereof. In the in vivo testing, high amount of new bone formation in the PCL/Mg hybrid porous scaffold was also found. These observations may further prove that the bone forming ability of biomaterials can be enhanced by the controlled release of magnesium ions in vivo. Indeed, the literatures reported that the presence of magnesium in the bone system was beneficial to bone growth and also played a key role in bone remodeling and skeletal development [5]. However, the amount of Mg ions released is critical for bone formation [6], as too

much Mg ions released would lead to bone loss. Hence, in addition to the improvement of mechanical properties of those scaffolds, the amount of Mg micro-particles incorporated into the scaffolds should be carefully controlled so as to avoid any adverse biological effect due to over release of magnesium ions [7].

SIGNIFICANCE: The incorporation of the magnesium micro-particles to pure PCL porous scaffold is able to improve its poor mechanical properties and inferior bioactivity. The controlled released of Mg ions can significantly enhance the bone formation ability of biomaterials in vivo.

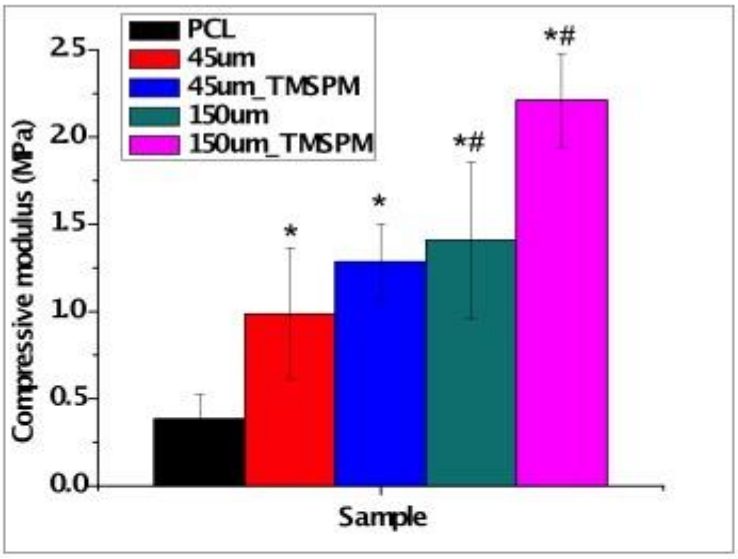

Figure 1 Compressive moduli of the pure PCL porous scaffold, Mg/PCL and silane-coupled Mg/PCL porous scaffolds. The compressive moduli of both the Mg/PCL and silane-coupled Mg/PCL scaffolds were found to be significantly higher (*$p<0.05$) than the pure PCL scaffold. In addition, the compressive moduli of the silane-coupled 150 μm Mg/PCL porous scaffolds was significantly higher ($^{\#}p<0.05$) than the 150 μm Mg/PCL scaffold.

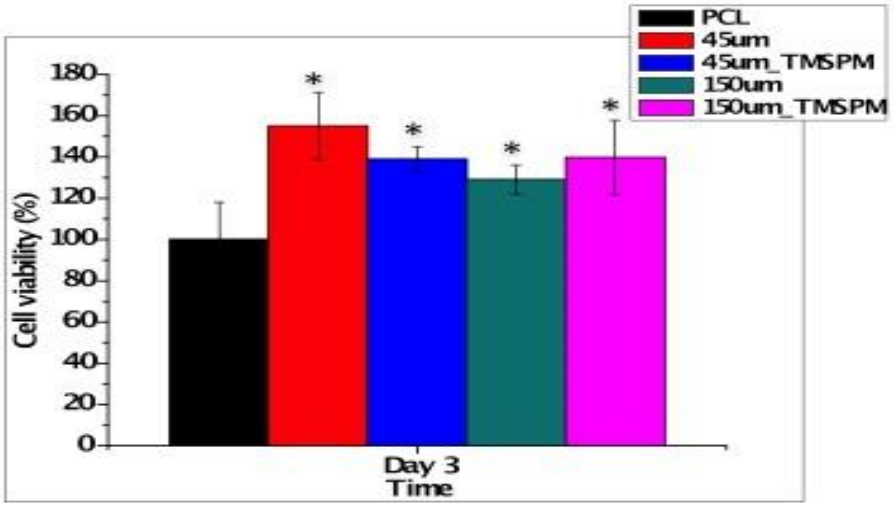

Figure 2 Cell viability of MC3T3-E1 pre-osteoblasts cultured on pure PCL, Mg/PCL and TMSPM silane-coupled porous Mg/PCL scaffolds. Cell viability was found significantly higher in both Mg/PCL and TMSPM silane-coupled porous scaffolds as compared to the pure PCL scaffold ($p<0.05$).

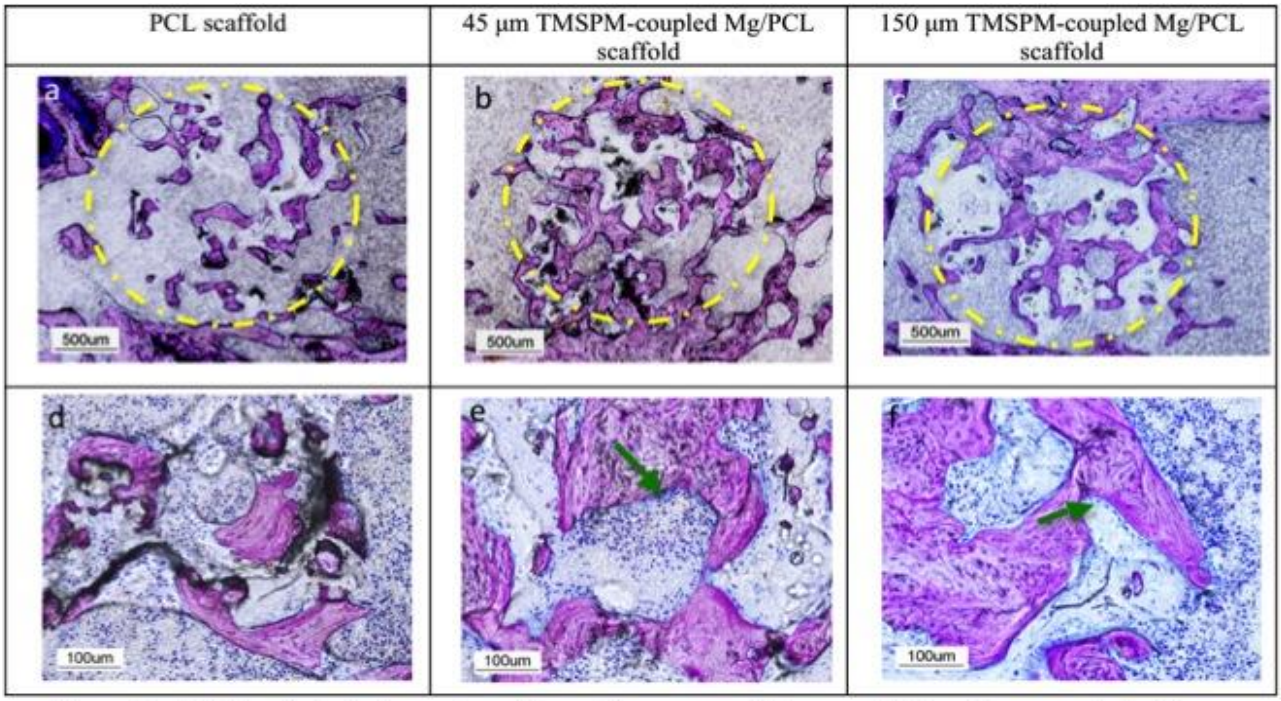

Figure 3 Histological photographs of bony tissue around the pure PCL, silane-coupled 45 μm particle Mg/PCL porous scaffold, and silane-coupled 150 μm particle Mg/PCL porous scaffold after 3 months of implantation. Fig. 3a–c showed the location of the scaffolds in yellow dotted line in 40x magnification. Fig. 3d–f revealed the newly formed bone and the presence of osteoblast-like cells (green arrows) within the scaffolds in 200× magnification.

Acknowledgements

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