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ORIGINAL ARTICLE

# Degradation and *in vitro* cell—material interaction studies on hydroxyapatite-coated biodegradable porous iron for hard tissue scaffolds

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**Summary** This paper describes degradation and cell—material interaction studies on hydroxyapatite (HA)-coated biodegradable porous iron proposed for hard tissue scaffolds. Porous iron scaffolds are expected to serve as an ideal platform for bone regeneration. To couple their inherent mechanical strength, pure HA and HA/poly(ε-caprolactone) (HA/PCL) were coated onto porous iron using dip coating technique. The HA/PCL mixture was prepared to provide a more stable and flexible coating than HA alone. Degradation of the samples was evaluated by weight loss and potentiodynamic polarisation. Human skin fibroblast (HSF) and human mesenchymal stem cells (hMSC) were put in contact with the samples and their interaction was observed. Results showed that coated samples degraded ~10 times slower (0.002 mm/year for HA/PCL-Fe, 0.003 mm/year for HA-Fe) than the uncoated ones (0.031 mm/year), indicating an inhibition effect of the coating on degradation. Both HSF and hMSC maintained high viability when in contact with the coated samples (100—110% control for hMSC during 2—5 days of incubation), indicating the effect of HA in enhancing cytocompatibility of the surface. This study provided early evidence of the potential translation of biodegradable porous iron scaffolds for clinical use in orthopedic surgery. However, further studies including *in vitro* and *in vivo* tests are necessary.


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## Introduction

Bone scaffolds have been used to facilitate the regeneration of new bone tissues and maintain a balance between temporary mechanical support, degradation, and cell growth. Separate from the widely chosen polymers, porous biodegradable metals have recently been viewed as potential materials for hard tissue scaffolds [1]. The inherent strength and ductility owned by metals are the key features that make them more appealing than polymers for hard tissue applications. Biodegradable metal scaffolds have shown interesting mechanical properties, especially Young's modulus and toughness, which are close to that of human bone with tailored degradation behaviour. For instance, the Young's modulus of magnesium (Mg; 41–45 GPa) is closer to that of cortical bone [2]. Despite having higher mechanical properties in fully dense state, (i.e., Young's modulus = 211 GPa), pure iron (Fe) strengths can be rendered having mechanical properties closer to those of bone by altering its porosity. Porous iron–phosphorus (Fe–P) alloys fabricated through powder metallurgy have resulted in an elastic modulus of 2.3 GPa, which is comparable to that of typical bone [3,4] More recently, porous pure Fe with a pore size of 450 um and 88% porosity exhibited a compressive strength of 0.33 MPa that falls within the range of cancellous bone strength [5–8]. In essence, the porosity and pore sizes of the porous biodegradable metals can be altered to obtain desired mechanical properties, degradation behaviour, and cell–material interaction. In addition, the porosity must be interconnected to provide spaces for osteogenesis while maintaining optimum mechanical properties of the scaffolds [9].

Mg and its alloys are the most studied biodegradable metals and the recent coating technology has enable Mg to have a more controllable degradation rate suitable for bone scaffold applications [10–14]. The porous structure of Mg has been proven to play a significant role in cell growth and proliferation [15,16]. Meanwhile, porous Fe was introduced much more recently [8,17,18]. The *in vitro* cytotoxicity test on three types of porous Fe manufactured by replication method, Fe–Mg, Fe, and Fe-carbon nanotubes, has shown proliferation of osteoblastic cells [18] and the degradation product of Fe was proven to be nontoxic to endothelial cells [19]. Despite its appealing *in vitro* cytocompatibility, the inherent high strength of Fe will easily provide the required initial strength of the scaffolds to stabilize the affected bone. Compared to Mg or polymeric scaffolds, higher strength of Fe will allow more flexible control on the porous structure to meet specific bone strength requirements [1,18].

Cell attachment, migration, differentiation, and proliferation in the porous structure are among the important cell–material interaction parameters that determine the suitability of a material for scaffolds. Starting with cell attachment, an attractive-to-cell surface should be prepared. Hydroxyapatite (HA) is a well-known bioactive ceramic material having similar chemical composition to human bone and has excellent bone bonding ability [20]. The use of HA coatings on metallic implants have been reported to stimulate faster bone cell attachment, resulting in an improvement healing rate and bone strength during the early stage of implantation [21,22]. However, HA alone is brittle and to overcome this issue, a coating of HA and poly(ε-caprolactone) (PCL) composite was proposed. This resulted in a more stable and flexible coating without cracking or delamination compared with the single HA coating [23]. This study will be the first to investigate cell–material interaction as valid evidence to propose HA-coated porous biodegradable iron as novel bone scaffolds. Samples of pure porous Fe, HA-coated porous Fe, and HA/PCL-coated porous Fe were tested for degradation and bioactivity. The interaction of human skin fibroblast (HSF1184) and human mesenchymal stem cells (hMSC) on the coated and uncoated samples were observed. Static immersion (weight loss) and potentiodynamic polarisation (PDP) tests were performed to investigate the degradation behaviour.

## Materials and methods

### Sample preparation

Interconnected-pores porous pure-Fe sheets (purity 99.9%, pore size = 450 μm, porosity = 88%) were kindly provided by Alantum Corporation, Korea. According to the manufacturer, the porous pure-Fe sheet was made via the polymer space holder method. Samples of the porous pure-Fe (10 mm × 10 mm × 1.6 mm) were coated with hydroxyapatite (HA, Sigma–Aldrich, USA) and composite of hydroxyapatite/poly(ε-caprolactone) (HA/PCL) using a dip coating method (Dip Coater PTL-MMB, MTI Corp, USA) to produce HA-coated porous Fe (HA-Fe) and HA/PCL-coated porous Fe (HA/PCL-Fe), respectively. The HA suspension were prepared by dissolving crystalline micro-HA powders in methanol at 10% weight/volume ratio at room temperature and homogeneously stirred for 72 hours. PCL pellets ($M_w$ = 65,000, Sigma–Aldrich) and HA powders (1:1 ratio) were dissolved in chloroform at 15% weight/volume ratio and then homogeneously stirred for 72 hours. The dip coating process was performed at a constant withdraw and down speeds of 200 mm/minute five times, and the coated samples were let to cure at room temperature for 24 hours [24]. Microstructure and surface morphology of the samples were analysed by a scanning electron microscope (SEM, Hitachi TM3000, Japan) coupled with energy-dispersive X-ray spectroscopy (EDX, SwiftED 3000, Oxford Instruments, UK).

### Degradation tests

Static immersion test were carried out in minimum essential medium solution (MEM, Gibco, Australia) similarly used for cell culture medium. Three samples of pure-Fe, HA-Fe, and HA/PCL-Fe were immersed in 100 mL MEM at 37°C for 7 days, 14 days, and 21 days in a $CO_2$ incubator. Weight of the samples was measured prior to and after the immersion test. The samples were rinsed in deionized water and ethanol and brushed gently as specified by the American Society for Testing and Materials (ASTM) G1-03 standard [25] followed by air and vacuum drying for 48 hours to completely remove all degradation products prior to weighing. Some samples were incubated for 25 days and