



## *In vitro* and *in vivo* bone formation potential of surface calcium phosphate-coated polycaprolactone and polycaprolactone/bioactive glass composite scaffolds



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### ABSTRACT

In this study, polycaprolactone (PCL)-based composite scaffolds containing 50 wt% of 45S5 Bioglass<sup>®</sup> (45S5) or strontium-substituted bioactive glass (SrBG) particles were fabricated into scaffolds using an additive manufacturing technique for bone tissue engineering purposes. The PCL scaffolds were surface coated with calcium phosphate (CaP) to enable further comparison of the osteoinductive potential of different scaffolds: PCL (control), PCL/CaP-coated, PCL/50-45S5 and PCL/50-SrBG scaffolds. The PCL/50-45S5 and PCL/50-SrBG composite scaffolds were reproducibly manufactured with a morphology highly resembling that of PCL only scaffolds. However, 50 wt% loading of the bioactive glass (BG) particles into the PCL bulk decreased the scaffold's compressive Young's modulus. Coating of PCL scaffolds with CaP had a negligible effect on the scaffold's porosity and compressive Young's modulus. When immersed in culture media, BG dissolution ions (Si and Sr) were detected for up to 10 weeks in the immersion media and surface precipitates were formed on both PCL/50-45S5 and PCL/50-SrBG scaffolds' surfaces, indicating good *in vitro* bioactivity. *In vitro* cell studies were conducted using sheep bone marrow stromal cells (BMSCs) under non-osteogenic or osteogenic conditioned media, and under static or dynamic culture environments. All scaffolds were able to support cell adhesion, growth and proliferation. However, when cultured in non-osteogenic media, only PCL/CaP, PCL/50-45S5 and PCL/50-SrBG scaffolds showed an up-regulation of osteogenic gene expression. Additionally, under a dynamic culture environment, the rate of cell growth, proliferation and osteoblast-related gene expression was enhanced across all scaffold groups. Subsequently, PCL/CaP, PCL/50-45S5 and PCL/50-SrBG scaffolds, with or without seeded cells, were implanted subcutaneously into nude rats for the evaluation of osteoinductivity potential. After 8 and 16 weeks, host tissue infiltrated well into the scaffolds, but no mature bone formation was observed in any scaffolds groups.

#### Statement of significance

This novelty of this research work is that it provide a comprehensive comparison, both *in vitro* and *in vivo*, between 3 different composite materials widely used in the field of bone tissue engineering for their bone regeneration capabilities. The materials used in this study include polycaprolactone, 45S5 Bioglass, strontium-substituted bioactive glass and calcium phosphate. Additionally, the composite materials were fabricated into the form of 3D scaffolds using additive manufacturing technique, a widely used technique in tissue engineering.

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## 1. Introduction

The self-healing capabilities of bone can be impeded beyond a critical size defect and require clinical intervention to reunite and regenerate the defect region. One promising approach to provide the supporting platform is the use of scaffolds fabricated via rapidly evolving 3D printing technologies [1]. These scaffolds can act as a temporary matrix to support/direct cellular growth/differentiation and to provide mechano-induction and ultimately bone remodelling. Presently, there are a wide variety of both materials and 3D printing technologies available for the fabrication of scaffolds [2]. Depending on the intended applications, the choice of materials and printing techniques may vary. In bone tissue engineering (TE), most polymer scaffolds undergoing clinical trials [3,4] lack osteoinductivity. Therefore, in the field of bone TE, the choice of biomaterials is moving towards composites to leverage the strength of individual materials and converge them into an advanced new biomaterial. For example, scaffold surfaces can be coated with appropriate inorganic materials that are largely calcium phosphate (CaP)-based to improve the bioactivity and effectiveness of a polymer scaffold's integration with host bone. Routinely, CaP-based biomaterials are used as a coating on metallic implants as they support bone growth along their surface when placed in the vicinity of host bone. This improves the osseous integration, prevents implant loosening, and reduces the risk for potential implant revision [5,6]. Coatings of polymer scaffolds with CaP have been widely explored and have been shown to promote osteoblast differentiation *in vitro* and/or bone formation *in vivo* [7–9]. Alternatively, bioactive materials such as CaP-based ceramics or bioactive glass (BG) can be incorporated into the polymer matrix. Due to their inherent bioactivity, such materials are widely used clinically for the repair of hard tissue in a variety of craniofacial, maxillofacial, and periodontal applications [10–12]. The combination of an inert polymer with bioactive materials can give rise to a bioactive composite [13–15]. Although many composites are currently being investigated for their potential in bone TE applications, there is a lack of studies that comprehensively compare the different composite materials. Therefore, in this study, a comprehensive comparison of both *in vitro* and *in vivo* conditions, between 3 different composite materials widely used in the field of bone tissue engineering for their bone regeneration capabilities will be presented.

In this study, we investigated the ability of polycaprolactone (PCL)-based bioactive composite scaffolds to promote cell growth, proliferation, and osteoblast differentiation *in vitro*. In our previous study [16], we found that the bioactive effect of BG (45S5 Bioglass® [45S5] or strontium-substituted bioactive glass [SrBG]) was hindered when loaded in low wt% (10 wt%) into PCL scaffolds, resulting in minimal effects on osteoblast differentiation compared to native PCL scaffolds [16]. In the current study, we incorporated 50 wt% of BG (45S5 or SrBG) into the PCL scaffolds and anticipated that higher loading of BG would further enhance the composite scaffolds bioactivity and improve osteoblast differentiation. To further elucidate the cellular response to polymer scaffolds coated with CaP compared to the proposed bioactive composite scaffolds, PCL scaffolds were coated with a layer of CaP.

It has been reported that under static culture conditions, cellular proliferation and viability are limited due to the inefficient exchange of nutrients and waste [17]. We therefore decided to use a bi-axially rotating bioreactor to increase cell density and improve cellular homogeneity throughout the scaffolds. Such a bioreactor creates a controllable and mechanically active environment that aids in the effective proliferation and growth of cells [18,19]. In the last experiment, we assessed the osteoinductive potential of the scaffolds described above using a subcutaneous rat model.

## 2. Methods and materials

### 2.1. Preparation of PCL/BG composite material

In this study, we used BG (45S5 and SrBG) with particle size  $\leq 20 \mu\text{m}$ . 45S5 with the composition of 46.13 SiO<sub>2</sub> – 2.60 P<sub>2</sub>O<sub>5</sub> – 24.35 Na<sub>2</sub>O – 26.91 CaO (mol%) and SrBG with the composition of 46.13 SiO<sub>2</sub> – 2.60 P<sub>2</sub>O<sub>5</sub> – 24.35 Na<sub>2</sub>O – 6.73 CaO – 20.18 SrO (mol%) [20] were incorporated into the PCL bulk by fast precipitation into excess ethanol [21]. Briefly, 10% (w/v) PCL solution was prepared by dissolving 10 g of PCL pellets (CAPA 6500, Perstorp, United Kingdom) in 100 ml of chloroform (MERCK Millipore, Australia) at room temperature. We next added 50 wt% of BG (45S5 or SrBG) relative to the PCL mass to the PCL solution and stirred until a homogenous mixture was achieved. The PCL/BG solution mixture was then precipitated into 5-fold excess of 100% ethanol (v/v) (MERCK Millipore, Australia). The solid PCL/BG composite was finally isolated and air-dried in order to evaporate the solvent.

### 2.2. Scaffolds fabrication

All scaffolds (PCL, PCL/50-45S5 and PCL/50-SrBG) were fabricated using melt extrusion based additive manufacturing (AM) technology [22] at 90 °C. All scaffolds were designed and fabricated using a 21G nozzle, with a lay-down pattern of 0–90°, filament gap of 2 mm and layer thickness of 0.4 mm. Scaffolds of 50 (L) × 50 (W) × 6 (H) mm or 50 (L) × 50 (W) × 2.4 (H) mm were fabricated and then cut to 6 × 6 × 6 mm or 4 × 4 × 2.4 mm dimensions, respectively. Scaffold characterisation and *in vivo* studies were undertaken using the 6 × 6 × 6 mm scaffolds while cell culture studies were done using the 4 × 4 × 2.4 mm scaffolds.

### 2.3. Calcium phosphate coating on PCL scaffolds

PCL scaffolds were coated with CaP using a method adapted from Vaquette et al. [7]. First, the fabricated scaffolds were immersed in 70% ethanol and placed under vacuum for 10 min to remove entrapped air bubbles that could have a negative impact on the coating homogeneity. Samples were then placed in a pre-warmed 37 °C, 5 M sodium hydroxide (NaOH) under vacuum for 10 min, and transferred to a 37 °C water bath for 1 h. The scaffolds were rinsed with MilliQ water to remove residual NaOH until the pH of the rinsing water was  $\sim$ pH 7. The scaffolds were then immersed for 1 h in filtered 10× simulated body fluid (SBF) adjusted to pH 6 with sodium bicarbonate (NaHCO<sub>3</sub>) (initially described by Kokubo et al. [23]) at 37 °C with one change of fresh SBF solution after 30 min. The samples were then rinsed twice with MilliQ water, and immersed in 0.5 M NaOH at 37 °C for 30 min to homogenise the coated CaP phase. Finally, the scaffolds were rinsed with MilliQ water until the rinsing solution reached  $\sim$ pH 7.

### 2.4. Surface morphology, porosity, and BG distribution

The surface morphology of the fabricated scaffolds was examined using scanning electron microscopy (SEM) (FEI Quanta SEM/FIB) at 10 kV after gold sputter-coating (Biorad SC500). Scaffold porosity and the distribution of BG particles within the scaffolds were examined using Micro-CT 40 scanner (Scanco Medical, Brüttisellen, Switzerland) at a voxel size of 6  $\mu\text{m}$ . Samples ( $n = 8$ ) were evaluated at a filter width of 1.0 pixels and filter support of 2.0 pixels.

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