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Improved dimensional stability with bioactive glass fibre skeleton in poly(lactide-co-glycolide) porous scaffolds for tissue engineering

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ABSTRACT

Bone tissue engineering requires highly porous three-dimensional (3D) scaffolds with preferable osteoconductive properties, controlled degradation, and good dimensional stability. In this study, highly porous 3D poly(D,L-lactide-co-glycolide) (PLGA) — bioactive glass (BG) composites (PLGA/BG) were manufactured by combining highly porous 3D fibrous BG mesh skeleton with porous PLGA in a freeze-drying process. The 3D structure of the scaffolds was investigated as well as *in vitro* hydrolytic degradation for 10 weeks. The effect of BG on the dimensional stability, scaffold composition, pore structure, and degradation behaviour of the scaffolds was evaluated. The composites showed superior pore structure as the BG fibres inhibited shrinkage of the scaffolds. The BG was also shown to buffer the acidic degradation products of PLGA. These results demonstrate the potential of these PLGA/BG composites for bone tissue engineering, but the ability of this kind of PLGA/BG composites to promote bone regeneration will be studied in forthcoming *in vivo* studies.

1. Introduction

Tissue engineering with highly porous three-dimensional (3D) biodegradable scaffolds has emerged as a promising method for bone tissue regeneration [1–4]. Especially polymer based scaffolds together with a ceramic phase acting as an osteoconductive component have been widely studied for bone regeneration [1,5]. A highly porous structure with open and interconnected pores is required for optimal tissue integration into the scaffolds after implantation [3]. For bone tissue engineering, the scaffold should also provide temporary mechanical support, osteoconductivity, controlled degradation rate, biocompatibility of the used materials and their degradation products, and be sterile [6].

Poly(lactide-co-glycolide) (PLGA) is the most studied biodegradable synthetic polymer for biomedical applications. It is widely used as sutures and drug delivery devices as it degrades rapidly compared to other biodegradable polyesters. Promising results have already been demonstrated with PLGA and it has also been approved by FDA (Food and Drug Administration) [7].

PLGA has been widely studied as tissue engineering scaffolds as well because it demonstrates favourable cell adhesion and proliferation properties [8,9]. Particularly, PLGA has been studied for use in porous tissue engineering scaffolds because of its tuneable degradation rate, good mechanical properties and processability [10]. Porous PLGA scaffolds often suffer from low mechanical strength and lack the osteoconductivity and hydrophilicity required for optimal bone tissue engineering [11].

Acidic by-products which result from PLGA degradation may lead to harmful pH decrease in the implantation site. Bioactive glass (BG), on the other hand, is hydrophilic by nature, possesses osteoconductive properties, and has good compression strength making it a good candidate for bone tissue engineering. Also, BG is shown to buffer the acidic degradation of PLGA [12]. This is why PLGA/BG composites are thought to overcome the limitations of plain PLGA scaffolds for improved bone regeneration.

Freeze-drying is a conventional method to fabricate porous tissue engineering scaffolds. Many of the previously studied freeze-dried polymer-bioactive ceramic scaffolds for bone tissue engineering have been prepared of bioactive ceramic filler particles and either natural polymers, such as collagen or chitosan [13–19], or synthetic polymers, such as PLGA or PDLLA [12,20–22]. The use of fibrous bioactive ceramic

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filler in a freeze-dried polymer matrix for bone tissue engineering scaffolds has as far as we know not been reported previously.

The potential of bioresorbable glass fibre-reinforced composites for load-bearing applications has been reported previously [23]. In the present study highly porous freeze-dried PLGA/BG composites for bone tissue engineering were prepared and studied. The aim was to develop highly porous three-dimensional composite scaffolds by incorporating porous PLGA with a highly porous BG fibre mesh skeleton in a freeze-drying process. Two PLGA–BG composite scaffolds were compared to two plain PLGA scaffolds. The manufactured scaffolds were studied 10 weeks *in vitro*. The dimensional stability, scaffold composition (TGA), scaffold pore structure (SEM, microCT), contact angle, ability of BG to buffer the acidic degradation products of PLGA, and degradation rates (GPC, DSC) of the scaffolds were studied.

2. Materials and methods

2.1. Materials

Medical grade D-lactide and glycolide monomers (purity of raw materials >99.5%) were purchased from PURAC Biochem bv (Gorinchem, the Netherlands) and L-lactide monomer (>99%) from Futerro (Escanaffles, Spain). Tin(II) 2-ethylhexanoate (approx. 95%), 1-decanol (99%, distilled prior to use), dichloromethane (≥99.9%), and 1,4-dioxane were purchased from Sigma-Aldrich (Helsinki, Finland).

Two compositions of bioresorbable melt-derived glass fibres (Vivoxid Ltd., Turku, Finland), denoted as BG1 and BG2 of a system Na_2O–CaO–MgO–Al_2O_3–B_2O_3–P_2O_5–SiO_2, were used as received as reinforcement for the composites. The fibres were coated fibres with a sizing layer constituted of 3-glycidoxypropyltriethoxysilane and low molecular weight polycaprolactone (PCL). The average diameter of the fibres was 13 μm. The compositions of BG1 and BG2 fibres are presented in Table 1.

2.2. Methods

2.2.1. Polymerization

PLGA with a rac-lactide-to-glycolide ratio of 70:30 was synthesized in an inert (argon) atmosphere by ring-opening polymerization. Briefly, 100 g L-lactide, 100 g D-lactide and 69 g glycolide were weighed into a round bottle at room temperature (RT). The monomers were freshly obtained and stored at −20 °C until use. The bottle was heated to 120 °C and kept at this temperature until all monomers were molten. After this, 0.1 mol-% of tin(II) 2-ethylhexanoate (initiator) and a molecular weight defining amount of 1-decanol (co-initiator) were added under initial heavy stirring. The temperature was raised to 150 °C and kept constant for 5 h. The reaction mixture was cooled to RT and subsequently dissolved in 2 L of dichloromethane and precipitated in a 6-fold amount of ethanol under vigorous stirring to remove unreacted monomers and other impurities. The polymer product was dried in vacuum at 40 °C for approximately one week until no residual solvent could be observed in ^{1}H-NMR. The dried polymers were cut to granules.

2.2.2. Scaffold fabrication

Two different molecular weight PLGAs, poly(D,L-lactide-co-glycolide) 70/30 were manufactured for matrix polymer. The weight average molecular weight (M_w) was 76 300 g/mol and 48 300 g/mol for PLGA1 and PLGA2, respectively.

BG1 and BG2 mesh were manufactured for the composite scaffolds. The glass fibres were cut to staple fibres (length of ~10 cm) and carded into mesh. The mesh was cut with a puncher to produce samples with a radius of 14 mm.

PLGA solutions of 3 and 5 wt.% were prepared by dissolving PLGA in 1,4-dioxane. The solutions were stirred vigorously overnight to form uniform solution. For plain PLGA scaffolds, 5 wt.% PLGA solution was poured into custom made Teflon sample moulds (diameter 15 mm, height 3 mm). For composite scaffolds, 3 wt.% PLGA solution was poured into moulds after which the BG mesh was thoroughly immersed into the solution. The samples were frozen at −30 °C for 24 h prior to 24 h freeze-drying. The samples were held under vacuum at RT for a minimum of 48 h and gamma sterilized at 25 kGy before characterization. Table 2 shows the different (fabricated) scaffold types.

2.2.3. Microstructure evaluation

The microstructure of the scaffolds was studied with a scanning electron microscope (SEM) (JEOL Ltd, Tokyo, Japan). The top surface and cross-section of the scaffolds were imaged. The samples were sputtered with gold prior to analysis. MicroCT imaging with MicroXCT-400 (Carl Zeiss X-ray Microscopy, Inc., Pleasanton, USA) was used to analyse the 3D structure of the scaffolds. To determine the pore structure (porosity, pore size, material thickness and pore size distribution) of the scaffolds, Fiji [24] with BoneJ [25] plugin, MATLAB (MathWorks Inc., Natick, MA, USA) and Avizo 9.0 software were used. BoneJ thickness function fits spheres inside the structure. The value of each voxel will be the diameter of the biggest sphere that includes the voxel. Obtained data can be used to calculate *e.g.* the mean thicknesses and porosities for certain size particles. Open porosities were calculated with a self-made MATLAB programme. Open porosity is considered as the pores that are accessible from outside of the scaffold. Open porosity has been calculated for particles of different sizes by using thickness data. Pore size distribution was determined by using Avizo. The tube voltage and voxel size were 40 kV and 2.2 × 2.2 × 2.2 μm^3, respectively. No filters were used.

2.2.4. Contact angle measurements

Contact angle of the dry scaffolds prior to hydrolysis was examined with a Theta optical tensiometer (Biolin Scientific, Västra Frölunda, Sweden) device. The measurements were done with deionized water, phosphate-buffered saline (PBS, pH 7.40) and with bovine blood (commercially available) ($n = 6$ for each scaffold type with each different solutions).

2.2.5. In vitro studies

In vitro degradation studies, timed at weeks 0, 2, 4, 6, 8 and 10, were carried out using six parallel samples, each half the size of the original freeze-dried sample (weight ca. 15 mg). PBS prepared as described by Shah et al. [26], with the standard volume of 10 ml buffer per scaffold (according to International Standard, ISO 15814:1999 [27]) was used. The pH of the buffer was measured weekly, using a Mettler Toledo MP225 pH metre (Mettler-Toledo GmbH, Schwerzenbach, Switzerland). The buffer solution was changed fortnightly or weekly if pH exceeded the given limits (7.35–7.45).

Table 1
Oxide compositions (mol-%) of the bioactive glass fibres (BG1 and BG2).

Glass	SiO_2	CaO	MgO	B_2O_3	Al_2O_3	P_2O_5	Na_2O
BG1	68.7 ± 1.0	13.3 ± 0.2	4.4 ± 0.1	0.9 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	11.9 ± 0.3
BG2	68.6 ± 1.0	9.3 ± 0.2	7.2 ± 0.1	1.8 ± 0.1		0.6 ± 0.1	12.5 ± 0.3