



Long-term in vitro degradation of PDLA/Bioglass[®] bone scaffolds in acellular simulated body fluid

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ABSTRACT

The long-term (600 days) in vitro degradation of highly porous poly(D,L-lactide) (PDLA)/Bioglass[®]-filled composite foams developed for bone tissue engineering scaffolds has been investigated in simulated body fluid (SBF). Foams of ~93% porosity were produced by thermally induced phase separation (TIPS). The degradation profile for foams of neat PDLA and the influence of Bioglass[®] addition were comprehensively assessed in terms of changes in dimensional stability, pore morphology, weight loss, molecular weight and mechanical properties (dry and wet states). It is shown that the degradation process proceeded in several stages: (a) a quasi-stable stage, where water absorption and plasticization occurred together with weight loss due to Bioglass[®] particle loss and dissolution, resulting in decreased wet mechanical properties; (b) a stage showing a slight increase in the wet mechanical properties and a moderate decrease in dimensions, with the properties remaining moderately constant until the onset of significant weight loss, whilst molecular weight continued to decrease; (c) an end stage of massive weight loss, disruption of the pore structure and the formation of blisters and embrittlement of the scaffold (evident on handling). The findings from this long-term in vitro degradation investigation underpin studies that have been and continue to be performed on highly porous poly(α -hydroxyesters) scaffolds filled with bioactive glasses for bone tissue engineering applications.

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1. Introduction

There has been much emphasis on the development of Bioglass[®]-filled poly(α -hydroxyester) composite scaffolds for use in bone tissue engineering [1–4]. These scaffolds have been tested both in vitro and in vivo, and their convenience based on their bioactive and biodegradable characteristics has been highlighted [4–8]. For tissue engineering applications it is important that the degradation characteristics of the scaffold enable the pore structure to be maintained during the period of cell infiltration and tissue growth, in addition to maintaining the mechanical properties and structural integrity. It is required that the scaffold degradation rate in vivo is similar to the rate of tissue formation [9] and that the three-dimensional space is replaced by new tissue. The degradation rate affects cell viability and the host tissue response. In some cases long-lasting implants based on poly(α -hydroxyesters) have been associated with delayed inflammatory responses at implanta-

tion sites [10]. Poly(α -hydroxyesters) degrade principally by hydrolysis of the hydrolytically unstable ester bonds to lactic acid, which can be removed from the body via metabolic pathways [11]. In general, the scaffold degradation rate and kinetics can be tuned by altering the composition and porosity of the scaffold structure. Poly(α -hydroxyesters) degradation behaviour is influenced by a number of factors, including device structure (scaffold wall thickness, porosity and pore size), molecular weight, co-polymer composition [12], hydrophilicity, addition of inorganic inclusions (in the case of composites) [1], degree of crystallinity, the glass transition temperature and the environmental conditions [9] (including medium, pH and temperature). The hypothesis that the presence of Bioglass[®] inclusions in composite scaffolds will alter the degradation kinetics of poly(α -hydroxyesters) by preventing or retarding autocatalysis has been investigated [8].

The long-term degradation of porous poly(α -hydroxyester) scaffolds and the effect of glycolide content (fabricated by compression moulding and particulate leaching) has been reported [12]. Several other degradation studies have been carried out on related scaffolds, including low M_w poly(D,L-lactide) (PDLA) foams modified with poly(ethylene oxide)-block-poly(PDLA) [13] and bioactive glass-filled poly(lactide-co-glycolide) (PLGA) of varying

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matrix compositions (fabricated by high pressure CO₂ processing) [1], as well as investigation of the short-term degradation of highly porous Bioglass[®]-filled poly(α -hydroxyesters) fabricated by thermally induced phase separation (TIPS) in phosphate-buffered saline [14,15]. To date, however, no long-term (e.g. >200 days) study has been undertaken to underpin the work of many research groups working on this family of highly porous bioactive composite scaffolds (PDLLA/Bioglass[®] composites).

For the first time, in this study the long-term degradation (to 600 days) and wet in vitro compressive mechanical behaviour of PDLLA/Bioglass[®] composite scaffolds have been investigated. The following properties of the scaffolds were measured as a function of degradation time: water absorption, weight loss, dimensional stability, compressive strength and modulus (at 37 °C under wet and dry states), pH of the medium, glass transition temperature (T_g), polymer molecular weight and pore morphology. In vitro studies were conducted dynamically in simulated body fluid (SBF), with the fluid being exchanged at selected time points to better represent the in vivo environment. The information gained is significant for applications in bone tissue engineering, as the scaffolds should not only have the appropriate mechanical properties to enable handling, implantation (and in some cases cell culture), but also should withstand time-dependent mechanical forces exerted in vivo, furthermore, the micro-stress environment has been shown to influence cell response [16].

2. Materials and methods

2.1. Materials

Racemic poly(D,L-lactide) (PDLLA) with an inherent viscosity of 1.62 dl g⁻¹ and density of 1.26 g cm⁻³ was obtained from Purac biochem (Gorinchem, The Netherlands) and used without further purification. Melt-derived 45S5 Bioglass[®] particles of chemical composition (wt.%) 45SiO₂, 24.5CaO, 6P₂O₅, 24.5Na₂O [17] were incorporated as the inorganic bioactive phase in the composites. The Bioglass[®] particles used had a particle size of <20 μ m and were of irregular shape, with a mean particle size of 4 μ m, density of 2.825 g cm⁻³ and surface area 2.7 m² g⁻¹ [18].

Neat PDLLA and PDLLA/Bioglass[®] composite foams, filled with 5 and 30 wt.% Bioglass[®], nominally referred to as 5BG and 30BG, respectively, were produced by low temperature TIPS, as described elsewhere [14]. The porosity of the neat polymer and composite foams has previously been reported as 93–94% [19]. Foams weighing ~50 mg and measuring 10 × 10 × 7 mm were cut using double-edged foil razor blades and their moderately denser top and bottom surfaces removed to present a homogeneous pore structure, as previously described [19]. Detailed characterisation of the microstructure of the scaffolds has been presented elsewhere [19].

2.2. Degradation study in simulated body fluid (SBF)

SBF was prepared following the protocol introduced by Kokubo et al. [20]. Prior to immersion in SBF, samples were sterilized by UV irradiation for 30 min in a laminar flow hood (samples were turned over after 15 min). An immersion step, whereby air was displaced within the foam samples, was a critical preconditioning for in vitro studies, otherwise the foams floated and the test fluids could not interact effectively with the bioactive glass and access the polymer. A schematic diagram for the immersion protocol is shown in Fig. 1. Samples were placed in 50 ml Falcon[™] tubes with modified cell strainers (100 μ m nylon membrane, Falcon[™]) press fitted into the tubes at the 37.5 ml mark to ensure that the foam was submerged. SBF was added to the 45 ml level, which meant that the

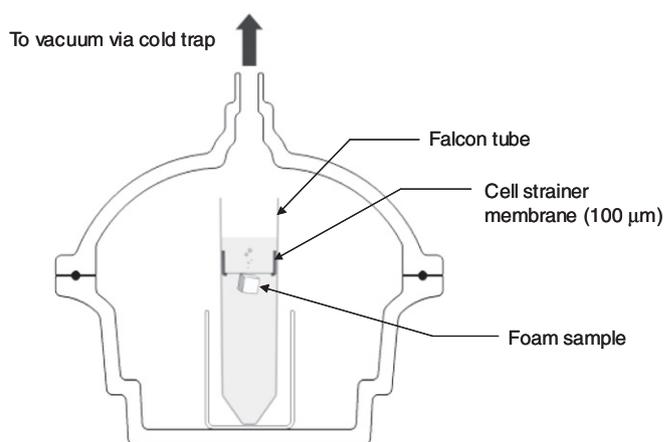


Fig. 1. Schematic diagram of the immersion set-up used to fully immerse the porous foams.

foams were entrapped by the membrane and maintained below the fluid level. Samples in tubes were placed inside a vacuum desiccator (previously sterilized with ethanol wipes) in a sterile laminar flow hood environment. A vacuum was applied to the desiccator using an Edwards M3 vacuum pump (Edwards, Crawley, UK) in line with a cold trap (immersed in liquid nitrogen), to prevent vapour entering the vacuum pump. Samples were kept under vacuum for 30 min, during which the fluids out-gassed and air was displaced from the foams (the solutions initially effervesced vigorously). After this period (effervescence was no longer observed) the vacuum desiccator was rapidly opened to the atmosphere, resulting in immediate sinking of the foams. The membrane prevented air at atmospheric pressure from re-entering the submerged foam on vacuum release.

Several different incubations in SBF were conducted in various experiments and for various time points up to 600 days in capped Falcon[™] tubes at 37 °C under tangential agitation of 175 rpm, using an orbital shaker (modified with multiple shelves) (C24 Incubator Shaker, New Brunswick Scientific). Foams were immersed individually in SBF solution, which was changed at selected time points as follows: 3 days, then weekly to 28 days, bi-monthly to 70 days, then every 8 weeks until termination of the study at 600 days. Three samples ($n = 3$) of each type (PDLLA, 5BG and 30BG) were evaluated in terms of morphological changes, changes in weight, thermal properties and degradation profile by size exclusion chromatography (SEC) at each time point. For mechanical evaluation eight samples for each selected time point were separately immersed in SBF.

2.3. Characterisation

2.3.1. Scaffold extraction from SBF

Samples were extracted from SBF at selected time points (2 and 24 h and 3, 7, 14, 21, 28, 37, 42, 70, 112, 300, 365, 450 and 600 days) and rinsed twice gently in deionized water (dH₂O). Dimensional changes were monitored by measuring the sample with Vernier calipers prior to immersion, on extraction (wet) and at selected time points after drying. Sample dimensions (width, length and height) were recorded at three places for each sample. Water absorption and weight loss were monitored during the degradation period.

2.3.2. Water absorption and weight loss

Water absorption was assessed by weighing samples prior to immersion, immediately post-incubation and after a period of blotting (60 min). Samples were blotted with laboratory tissue

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