Acta Biomaterialia 7 (2011) 2637–2643

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Evaluation of 3-D bioactive glass scaffolds dissolution in a perfusion flow system with X-ray microtomography

Sheng Yue, Peter D. Lee, Gowsihan Poologasundarampillai, Julian R. Jones *

Department of Materials, Imperial College London, London SW7 2AZ, UK

ARTICLE INFO

Article history:
Received 24 October 2010
Received in revised form 30 January 2011
Accepted 7 February 2011
Available online 26 February 2011

Keywords:
Scaffold
Image analysis
Porosity
Bioactive glass
Dissolution

ABSTRACT

Bioactive glass has high potential for bone regeneration due to its ability to bond to bone and stimulate osteogenesis whilst dissolving in the body. Although three-dimensional (3-D) bioactive glass scaffolds with favorable pore networks can be made from the sol–gel process, compositional and structural evolutions in their porous structures during degradation in vivo, or in vitro, have not been quantified. In this study, bioactive glass scaffolds were put in a simulated body fluid flow environment through a perfusion bioreactor. X-ray microtomography (μCT) was used to non-destructively image the scaffolds at different degradation stages. A new 3-D image processing methodology was developed to quantify the scaffold's pore size, interconnect size and connectivity from μCT images. The accurate measurement of individual interconnect size was made possible by a principal component analysis-based algorithm. During 28 days of dissolution, the modal interconnect size in the scaffold was reduced from 254 to 206 μm due to the deposition of mineral phases. However, the pore size remained unchanged, with a mode of 682 μm. The data presented are important for making bioactive glass scaffolds into clinical products. The technique described for imaging and quantifying scaffold pore structures as a function of degradation time is applicable to most scaffold systems.

1. Introduction

Synthetic bone grafts (scaffolds) are needed that can regenerate diseased or damaged bone, replacing the need for autografts. Autografts are the current gold standard whereby bone is transplanted, usually from the pelvis. Their disadvantage is the lack of supply and donor site morbidity, which can cause pain and other complications. Scaffolds are also vital components in bone tissue engineering strategies, where the aim is to culture cells on scaffolds prior to implantation. Scaffolds for bone regeneration are designed to act as temporary templates for bone and blood vessel growth. To do this they require a three-dimensional (3-D) porous network [1–3] with high porosity and connectivity for solute transport; with suitable pore and, more importantly, interconnect size distributions to enable cell migration, bone ingrowth and vascularization. The minimum interconnect diameter for human bone ingrowth is generally considered to be 100 μm [4,5]. The scaffold should also form a direct bond to the host bone by enabling cell adhesion and activity. Ideally the scaffold would degrade as the host bone forms, with the degradation products encouraging osteogenesis [2,6]. With all these different criteria to match, a method for quantifying degradation in vitro is needed as a first screening tool for new scaffolds.

Bioactive glasses are promising materials for bone tissue engineering scaffold fabrication [6,7]. They can bond to bone, degrade in the body and release ions which stimulate bone regeneration. When implanted in the body, bioactive glasses form a surface layer of crystalline hydroxyl carbonate apatite (HCA) [8–10], which is similar to bone mineral and is responsible for glass bonding with bone. The osteogenic property of these glasses has been attributed to the release of soluble silica and calcium ions during dissolution [6]. Using a sol–gel foaming process, 3-D bioactive glass scaffolds can be fabricated from bioactive glasses [11], achieving compressive strengths [2,12] and pore structures similar to human trabecular bone, with macropore diameters of 300–600 μm and interconnect diameters in excess of 100 μm [13]. Having achieved structural similarity, quantifying their dissolution behavior is another essential step in their development.

X-ray microtomography (μCT, micro-CT or XMT) can non-destructively image scaffolds in three dimensions [14,15]. Coupled with imaging processing, μCT has become an established tool for scaffold quantification [16–19]. For example, the internal pore structure can be characterized from μCT images, providing quantitative information such as: percentage porosity, pore and interconnect size distributions, network connectivity, pore shape and strut thickness [13,20–22]. The interaction between the scaffold material and its host environment, including bone ingrowth, has also been observed and quantified with μCT, both in vivo and ex vivo [20,22–24]. Furthermore, the non-destructive nature of μCT can

* Corresponding author. Tel.: +44 20 75946749; fax: +44 20 75946757.
E-mail address: julian.r.jones@imperial.ac.uk (J.R. Jones).

1742-7061/$ - see front matter © 2011 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2011.02.009

enable the evaluation of samples in situ, where experiments such as compression and mineral deposition are performed in vitro, whilst non-invasively scanning the scaffold [25–27]. In addition, the 3-D scaffold images can be used to generate meshes for computational fluid dynamic and mechanical simulations [13,28,29]. Therefore, many aspects of the performance of scaffolds can be evaluated and then improved in a non-invasive and quantifiable manner, reducing product design costs and time.

Current bioactivity and degradation tests for bioceramics usually consist of immersing a sample in a fixed volume of simulated body fluid (SBF), then placing it in an incubator and observing the final extent of HCA formation. However, bioactive glasses, and many other bioceramics, are dynamic materials which undergo dissolution before a HCA layer can deposit on their surface. A test that quantifies this precursor stage is required. For example, a high glass concentration can cause calcite to deposit on the glass surface instead of HCA [30]. The in vivo environment is not a closed environment and saturation of ions is not expected. Therefore, the prescreening process for new scaffolds needs improvement. Perfusion bioreactors are widely used in vitro in tissue engineering applications because perfusion flow can enhance mass transport and introduce shear stress, thereby improving tissue growth [31]. The aim of this work is to use a perfusion bioreactor with a local inhomogeneous flow rate, combined with μCT imaging and image analysis, to study the degradation behavior of bioactive glass foam scaffolds in an SBF flow environment. In order to achieve this, new and improved algorithms were needed with better accuracy and greater automation (using a principal component analysis (PCA)-based approach) than those developed previously for scaffold quantification [13,21]. The hypothesis was that the porous scaffold would undergo dissolution over time, causing the interconnects of the pores to open. Secondary objectives were to determine whether the deposition of the HCA layer could be observed using this technique; whether changes in pore size could be measured; and whether preferential flow within the scaffold would cause heterogeneous dissolution.

2. Materials and methods

2.1. Bioactive glass scaffolds synthesis

Bioactive glass foams of 70S30C composition (70 mol.% SiO_2, 30 mol.% CaO) were prepared using a sol–gel foaming method as previously described [11,12]. The sol preparation began with mixing 0.2 N nitric acid with deionized water using a magnetic stirrer, followed by addition of tetraethyl orthosilicate (TEOS) and calcium nitrate (all Sigma–Aldrich) in order. The initial molar ratio of water to TEOS (*R* ratio) was 12:1. Aliquots of 50 ml of the sol were combined with 3 ml of 5 vol.% hydrofluoric acid (HF, catalyst) and 0.35 ml of Teepol (surfactant, Thames Mead Ltd, London), then foamed using vigorous agitation. As the foamed sol approached the gelation point, it was cast into cylindrical Teflon® moulds, sealed and aged at 60 °C for 72 h. Previous work has shown that Teflon® moulds are non-reactive and facilitate removal, as well as improving the homogeneity of the glass composition [32]. The samples were then dried in air for a total of 94 h at 60, 90 and 130 °C. Finally, the scaffolds were stabilized at 600 °C for 3 h and then sintered at 800 °C for 2 h. The thermal processing was optimized in previous studies, with the drying process designed to prevent cracking and the sintering used to improve compressive strength [12].

2.2. Perfusion bioreactor system

An SBF solution, with ion concentrations similar to those of human extracellular fluid, was prepared following the Kokubo method [33]. Cylindrical bioactive glass scaffolds, 6 mm in diameter and 7 mm high and weighing 0.13 ± 0.2 g, together with a length of non-degradable silica glass fibre, were wrapped with Teflon tape and inserted into a Teflon® ring (termed a *scaffold assembly*). (Note that the silica glass fibre was added to calibrate the X-ray attenuation in the μCT, enabling the change in density of the scaffold to be monitored). The Teflon® ring containing the sample was then placed into the cylindrical chamber of a perfusion bioreactor (Gradient Container, Minucells Minutissue, Weinheim, Germany [34]), as illustrated in Fig. 1. In order to prevent flow from bypassing the scaffold, the outer diameter (12 mm) of the Teflon® ring matched the inner diameter of the chamber, while the Teflon® ring was also wrapped with Teflon® tape to ensure a tight seal. Three samples were run in separate experiments on separate occasions using fresh SBF, but using the same perfusion chamber.

The SBF flow circulation, which was directed through the scaffold from bottom to top with a flow rate of 1 ml min^{-1}, was maintained with a peristaltic pump (Masterflex, model 07519–25, Cole-Palmer Instrument Co., Niles, IL, USA). A 1 l reservoir of SBF was maintained at 37 °C in an incubator.

2.3. Optical emission inductive coupled plasma spectroscopy

For each of the three samples, at dissolution time points of 0, 1, 8, 24 h, 7 days and 28 days, 50 ml of the SBF solution was collected for analysis. The entire SBF was replaced after 7 days. The ion concentrations of P, Si and Ca in the collected solutions were measured with optical emission inductive coupled plasma (ICP) spectroscopy (Thermo Scientific ICP Spectrometer, Model iCAP 6300 Series Duo).

2.4. X-ray microtomography

μCT was used to scan the scaffold assembly as a function of degradation time in SBF flow. Each scaffold assembly was scanned before the dissolution study using a lab-based μCT unit (Phoenix X-ray Systems and Services GmbH, Wunstorf, Germany) at 100 kV and 100 μA, and with a voxel size of 8 μm. At 24 h, 7 days and 28 days, the dissolution experiment was paused and the scaffold assembly was removed out from the bioreactor, dried at 37 °C overnight and rescanned by μCT with the same setting as that of the 0 h scan. After μCT scanning, the assembly was reinstalled and the dissolution experiment resumed.

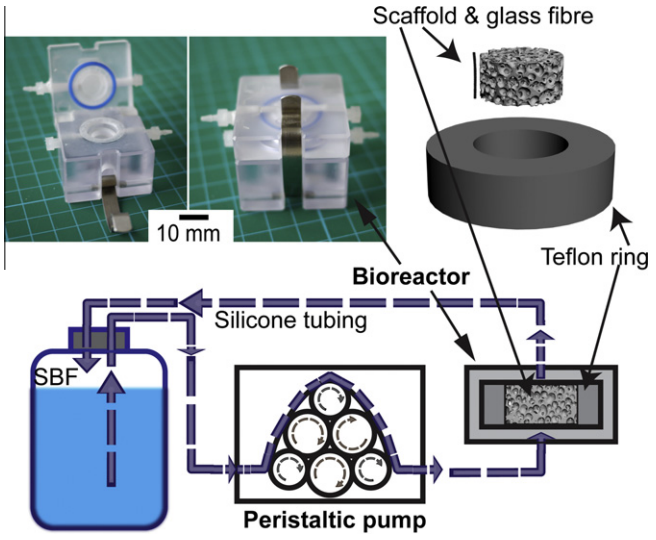

Fig. 1. Schematic of the dissolution experimental setup. The arrows on the silicone tubing indicate the SBF flow direction.

ID	Title	Pages
1372	Evaluation of 3-D bioactive glass scaffolds dissolution in a perfusion flow system with X-ray microtomography	7