Acta Biomaterialia xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Cotton-wool-like bioactive glasses for bone regeneration

G. Poologasundarampillai [a,*], D. Wang [a], S. Li [a], J. Nakamura [b], R. Bradley [c], P.D. Lee [c], M.M. Stevens [a,d], D.S. McPhail [a], T. Kasuga [b], J.R. Jones [a]

[a] Department of Materials, Imperial College London, South Kensington, London SW7 2AZ, UK
[b] Department of Frontier Materials, Nagoya Institute of Technology, Nagoya 466-8555, Japan
[c] School of Materials, The University of Manchester, Oxford Rd., Manchester M13 9PL, UK
[d] Department of Bioengineering, Imperial College London, South Kensington, London SW7 2AZ, UK

ARTICLE INFO

Article history:
Received 12 November 2013
Received in revised form 13 May 2014
Accepted 16 May 2014
Available online xxxx

Keywords:
Electrospinning
Sol–gel
Bone regeneration scaffold
3-D cotton-wool-like structure
Inorganic fibers

ABSTRACT

Inorganic sol–gel solutions were electrospun to produce the first bioactive three-dimensional (3-D) scaffolds for bone tissue regeneration with a structure like cotton-wool (or cotton candy). This flexible 3-D fibrous structure is ideal for packing into complex defects. It also has large inter-fiber spaces to promote vascularization, penetration of cells and transport of nutrients throughout the scaffold. The 3-D fibrous structure was obtained by electrospinning, where the applied electric field and the instabilities exert tremendous force on the spinning jet, which is required to be viscoelastic to prevent jet break up. Previously, polymer binding agents were used with inorganic solutions to produce electrospun composite two-dimensional fibermats, requiring calcination to remove the polymer. This study presents novel reaction and processing conditions for producing a viscoelastic inorganic sol–gel solution that results in fibers by the entanglement of the intermolecularly overlapped nanosilica species in the solution, eliminating the need for a binder. Three-dimensional cotton-wool-like structures were only produced when solutions containing calcium nitrate were used, suggesting that the charge of the Ca^{2+} ions had a significant effect. The resulting bioactive silica fibers had a narrow diameter range of 0.5–2 μm and were nanoporous. A hydroxycarbonate apatite layer was formed on the fibers within the first 12 h of soaking in simulated body fluid. MC3T3-E1 preosteoblast cells cultured on the fibers showed no adverse cytotoxic effect and they were observed to attach to and spread in the material.

1. Introduction

Bone tissue regeneration strategies aim to use synthetic temporary templates (scaffolds) to aid the natural healing of bone defects. Osteoblasts secrete bone extracellular matrix (ECM), which is composed of collagen fibrous structure, with mineralized calcium phosphate [1,2]. Therefore, in defects where load-bearing materials are not needed, an ideal biomaterial scaffold for bone regeneration might have a three-dimensional (3-D) fibrous structure that mimics the ECM [3–5] and can be easily pushed into position by a surgeon or dentist. The scaffolds are also required to be biocompatible, bioactive (bond with bone) and bioresorbable [6]. Bioactive glasses can form a rapid bond with bone through formation of a hydroxycarbonate apatite (HCA) surface layer on contact with body fluid, and through release of soluble silica and calcium ions that can stimulate osteoprogenitor cells to produce more bone [7].

Electrospinning is a versatile technique for producing continuous fibers with diameters ranging from nano- to micrometer, mimicking the fibers of ECM [8–10]. The high porosity and large surface area of the electrospun nanofibrous material provide numerous binding sites for protein adsorption and cell attachment [11]. Non-woven, aligned, crossed, layered and coaxial nanofibers with a high surface area can be produced by controlling the electrospinning parameters [9,12,13]. Nanofibers of numerous organic polymers have been produced [10] because of the ease of formation of a viscoelastic solution in a volatile solvent that can be easily electrospun.

Electrospun polymer fibermats have applications as vascular grafts and scaffolds for nerve, bladder matrix, lung and bone regeneration [14,15], using primarily bioresorbable and degradable natural and synthetic polymers [14,16–21]. However, bioactivity and enhancement of osteogenesis by the release of calcium ions and silica species are not provided by polymers alone.

The sol–gel process has been used to produce bioactive glass foam scaffolds for bone repair [22–24]. Recently, hydroxyapatite and silica-based bioactive and bioresorbable inorganic nanofibers

* Corresponding author. Tel.: +44 (0)75 0804 8322; fax: +44 (0)20 7594 6757.
E-mail address: gowsh.p@imperial.ac.uk (G. Poologasundarampillai).

http://dx.doi.org/10.1016/j.actbio.2014.05.020
1742-7061/

have been electrospun for biomedical applications from sol–gel solutions [25–31]. Kim et al. [26] and later Lu et al. [32] were the first to successfully electrospin silica-based bioactive glass nanofibers from a sol–gel solution, using polyvinylbutyral as the binder. Post-spinning, the fibermat was heat-treated at 700 °C to eliminate the organic phase. Further, Kim et al. [26] showed in vitro attachment and differentiation of bone marrow-derived stem cells on the bioactive nanofibrous scaffold and compared it with bioactive glass films of the same composition. Cells cultured on the nanofibers showed enhanced ALP expression compared with those grown on discs of the same glass.

So far, all the electrospun bioactive sol–gel-derived nanofibers reported have been produced using a polymer binding agent; the calcination of the fibers at high temperature leads to embrittlement and partial crystallization. However, Ma et al. [33] produced bioactive core–shell structured fibermats through a two-step method without using a polymer binding agent. First, they electrospun silica fibers [34], which were then coated with a bioactive glass shell by immersion in a modified Stöber sol.

Structurally, all the inorganic fibermats reported in the literature were two-dimensional (2-D) with small inter-fiber spacing. Three-dimensional scaffolds are necessary to act as templates and regenerate bone in large defects, and it is estimated that the interconnected spaces >100 μm are required for vascularized bone tissue growth [35]. The fiber–fiber separation distance (pore size) in a fibermat is related to the fiber diameter, where larger fiber diameter leads to larger fiber–fiber separation distances. For a fibermat, fiber diameters >∼20 μm are recommended to create a stable mat with a pore size >100 μm [36]. These fibers are approximately the diameter of an osteoblast, and therefore the cells interact with the fibermat as they would on solid material. Therefore, such mats do not mimic the ECM.

The aim here was to develop an electrospun bioactive glass scaffold with smaller fiber diameters in the range 0.3–1 μm [4,11], with a 3-D macroporous architecture. In electrospinning, the term "3-D" is often associated with 2-D fibermats that are thicker (∼0.5–2 mm) than conventional fibermats (<0.5 mm) [37–39]. This does not increase the inter-fiber distance of the fibermat. Here, it is important to define the cotton-wool-like structured materials as a separate and superior class of 3-D scaffolds. Recently, melt-derived Bioglass fibres were produced by laser spinning [40], and work by Mo-Sci Corp in the USA recently showed that melt-derived "cotton-candy" borate fibers produced good clinical results, healing chronic diabetic foot ulcers [41]. The inventors used melt blowing to produce fibers with a range of fiber diameters for wound regeneration. For bone regeneration, Stark et al.'s group [42] was one of the first to use the term "cotton-wool-like" for electrospun fibers of poly(lactide-*co*-glycolide)/amorphous tricalcium phosphate nanocomposite material. Later, Obata et al. [43] also electrospun poly(L-lactic acid) and siloxane-doped vaterite (SiV) composite cotton-wool-like fibers. Both had a structure similar to cotton-wool, but they both employed a post-electrospinning process to produce the cotton-wool-like structure: a manual fiber unravelling method in the case of Schneider et al. [42] and a fan method in the case of Obata et al. [43].

In the present work, the sol–gel processing was used in combination with electrospinning to produce the first bioactive sol–gel silica 3-D cotton-wool-like structured scaffolds for bone regeneration.

2. Materials and methods

The silica precursor tetraethylorthosilicate (TEOS) was purchased from Sigma–Aldrich, and all other chemicals for the synthesis of the sol–gel electrospun fibers were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

2.1. Fiber production

2.1.1. Sol–gel precursor solution preparation

The hydrolysis and condensation of TEOS to Si–O–Si linear chains under acid catalysis with an R ratio (H_2O:TEOS) of 2 has been reported by several groups for drawing silicate fibers [44–46]. Following this method, 100 mol.% SiO_2 (100S) precursor solutions for electrospinning were first prepared by mixing, in the order TEOS, ethanol, water and 1 N HCl to a final molar ratio of TEOS:ethanol:water:HCl of 1:2:2:0.01. The mixture was stirred for 24 h at room temperature. Calcium-containing fibers with a nominal composition of 70 mol.% SiO_2 and 30 mol.% CaO (70S30C) were prepared by the addition of calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) after 1 h of mixing TEOS, ethanol and 1 N HCl. A final ratio of TEOS:ethanol:water:HCl:$Ca(NO_3)_2$ of 1:2:2.23:0.01:0.3 was obtained. The 70S30C precursor solution was also left to react (age) for 24 h then heated at 70 °C in an oven, while continuously mixing to increase the viscosity of the sol. The solution viscosities of 100S and 70S30C were measured before and after the evaporation of ethanol, using a TOKI, RE-80H cone rotor viscometer. Ten viscosity measurements were performed in 5 min on 1 ml of solution, and the mode was selected as the actual viscosity. Table 1 lists the quantities of the reagents used and the amount of solvent evaporated. After the evaporation of ethanol, the sol–gel precursor solutions were loaded into a metallic needle (22 gauge), attached to a glass syringe and spun on the Nanofiber Electrospinning Unit (NEU, Kato Tech Co, Japan). The high-tension field was applied to the metal needle. The fibers were collected on Teflon-coated aluminum foil placed on a rotating drum that was positioned at a distance of 100 mm from the capillary. The electrospinning conditions used for spinning both solutions are listed in Table 1. The electrospun fibers were then transferred to an oven and heat-treated at 60 °C for 72 h.

Images and videos of electrospinning were obtained using an Olympus Pen lite E-PL1 camera equipped with 60× zoom and shutter speeds of 1/2000 s. Brightness and contrast of the photos were enhanced in ImageJ.

2.2. Characterization

2.2.1. Morphology and physical structure

Low magnification images of the fibers were produced by scanning electron microscopy (SEM; JSM-6301). Samples were coated with amorphous osmium using an Os coater and observed under 5 kV accelerating voltage and 15 mm working distance. High-magnification images were obtained by SEM on a LEO-1525 microscope equipped with a GEMINI field emission column. Samples were sputter coated with chromium to a maximum coating thickness of 13 nm before imaging. An operating voltage of 5 kV and working distances of 5–8 mm were used. Transmission electron microscopy (TEM) images were collected on a JEOL 2010 equipped with a LaB_6 filament. An operating voltage of 200 kV was used on samples thinner than 100 nm, prepared using a focused ion beam (FIB; Helios NanoLab 600) miller. FIB was used to section thin slices from 70S30C samples embedded in epoxy resin.

2.2.2. Chemical and atomic structure

A time-of-flight secondary ion mass spectroscopy (TOF-SIMS) instrument (ION–TOF GmbH, Germany) was applied to determine the distribution of silicon and calcium along the electrospun 70S30C bioactive glass fibers. A 25 keV Bi_3^+ primary ion source with a current of 0.1 pA was used to construct the secondary ion maps (512 × 512 pixels) on a 130 × 130 μm^2 area at an accumulated ion dose intensity of 1.21×10^{13} ions cm^{-2}. A low-energy 20 eV electron flood gun was used for charge compensation. Attenuated total reflectance–Fourier transform infrared spectroscopy (ATR-FTIR), X-ray diffraction (XRD), ^{29}Si magic angle spinning nuclear

Please cite this article in press as: Poologasundarampillai G et al. Cotton-wool-like bioactive glasses for bone regeneration. Acta Biomater (2014), http://dx.doi.org/10.1016/j.actbio.2014.05.020