

Simultaneous electrospin–electrosprayed biocomposite nanofibrous scaffolds for bone tissue regeneration

Lijo Francis^{a,b}, J. Venugopal^{a,*}, Molamma P Prabhakaran^a, V. Thavasi^a, E. Marsano^b, S. Ramakrishna^{a,c}

^a Nanoscience and Nanotechnology Initiative, Faculty of Engineering, National University of Singapore, Singapore

^b Department of Chemistry and Industrial Chemistry, University of Genova, Genova, Italy

^c King Saud University, 11451 Riyadh, Kingdom of Saudi Arabia

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ABSTRACT

Currently, the application of nanotechnology in bone tissue regeneration is a challenge for the fabrication of novel bioartificial bone grafts. These nanostructures are capable of mimicking natural extracellular matrix with effective mineralization for successful regeneration of damaged tissues. The simultaneous electrospinning of nanohydroxyapatite (HA) on electrospun polymeric nanofibrous scaffolds might be more promising for bone tissue regeneration. In the current study, nanofibrous scaffolds of gelatin (Gel), Gel/HA (4:1 blend), Gel/HA (2:1 blend) and Gel/HA (electrospin–electrospray) were fabricated for this purpose. The morphology, chemical and mechanical stability of nanofibres were evaluated by means of field emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy and with a universal tensile machine, respectively. The *in vitro* biocompatibility of different nanofibrous scaffolds was determined by culturing human foetal osteoblasts and investigating the proliferation, alkaline phosphatase (ALP) activity and mineralization of cells. The results of cell proliferation, ALP activity and FESEM studies revealed that the combination of electrospinning of gelatin and electrospinning of HA yielded biocomposite nanofibrous scaffolds with enhanced performances in terms of better cell proliferation, increased ALP activity and enhanced mineralization, making them potential substrates for bone tissue regeneration.

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1. Introduction

An important challenge in the field of bone tissue regeneration is the development of synthetic bone graft substitutes that are “intelligent” to instruct the *in vivo* environment to form bone [1]. Tissue engineering (TE) has emerged as a new multidisciplinary field that combines the latest developments in cell and molecular biology, materials science and engineering, chemistry and medical sciences in the development of hybrid substitutes (composed of biodegradable supports, cells, molecules and growth factors) aimed at restoring tissues or organ functions, using the natural signalling pathways and components of the organism [2]. Different results have shown that TE-based strategies having the potential to be used for the regeneration of a series of tissues and organs are especially suitable for the regeneration of bone tissues [3]. Bone tissue regeneration remains an important challenge in the field of orthopaedic and craniofacial surgery. Spinal fusions and repair of bone defects caused by trauma, tumours, infections, biochemical disorders and abnormal skeletal development are some examples of surgeries frequently performed in the clinic. In

most of these surgeries, there exists a great need for grafting materials [1]. The ultimate goal in scaffold design involves the fabrication of a suitable structure that can replace natural extracellular matrix (ECM) until the host cells can repopulate and resynthesize a new natural matrix [4].

Many types of materials have been proposed for both cartilage and bone TE, most of which are biocompatible and biodegradable polymers that follow previous applications in surgical procedures or other biomedical applications. Materials can be categorized as (i) natural polymers, (ii) synthetic polymers or (iii) ceramics, glasses and composites. Many techniques have been used to produce scaffolds for bone and cartilage TE; they include: solvent casting and particulate leaching [5–7], phase inversion [8–10], fibre bonding [11–13], melt-based technologies [14,15], membrane lamination [16], high-pressure-based methods [17], supercritical fluid technologies [18], freeze drying [19,20] and rapid prototyping techniques/free-form fabrication [21–24]. Polymeric nanofibrous scaffolds have huge potential for bone tissue engineering and should be biocompatible with the surrounding biological fluids and tissues, biodegradable and highly porous, with interconnected spaces, favourable for the diffusion of nutrients as well as the migration of large number of cells. Regular interconnected pores provide spacing for the vasculature required to nourish new bone

* Corresponding author. Tel.: +65 6516 4272; fax: +65 6773 0339.

E-mail address: nnijrv@nus.edu.sg (J. Venugopal).

and remove waste products. The present work describes a reliable and efficient method designed to fabricate biocomposite nanofibrous scaffolds by using simultaneous electrospinning (of gelatin) and electrospinning (of nanohydroxyapatite, HA) followed by cross-linking. The *in vitro* biocompatibility of fabricated biocomposite nanofibrous scaffolds was investigated by culturing human foetal osteoblasts (hFoB) on “electrospin–electrosprayed” nanofibrous scaffolds and compared to electrospun Gel/HA nanofibrous scaffolds for bone tissue regeneration.

2. Materials and methods

2.1. Materials

hFoB cells were obtained from the American Type Culture Collection (ATCC, Arlington, VA). Dulbecco's modified Eagle's medium/F-12 (HAM), foetal bovine serum (FBS), antibiotics and trypsin–ethylenediaminetetraacetic acid (EDTA) were purchased from Gibco Invitrogen, USA. Porcine gelatin was purchased from Sigma Aldrich. Crystalline HA was generously provided by the Department of Metallurgical and Materials Engineering, Indian Institute of Technology, Chennai, India. 2,2,2-Trifluoroethanol (TFE) was purchased from Fluka Chemicals Ltd., UK and glutaraldehyde was purchased from Sigma Aldrich, Singapore.

2.2. Electrospinning of nanofibrous scaffolds

Gelatin was dissolved in 10% (w/v) TFE solvent and electrospun at 9.5 kV (High voltage system, Gamma High Voltage Research, FL, USA), with the collector placed at a distance of 12 cm from the needle tip to collect the nanofibres. A specific amount of HA was dispersed in TFE, sonicated for 1 h and mixed with gelatin in the TFE solution. The weight ratios between the gelatin and HA in the blend solutions were optimized to 80:20 (4:1) and 80:40 (2:1), and the total concentration of the solution was maintained at 10% (w/v). The blend solutions were fed into a 5 ml standard syringe attached to 22 G blunted stainless steel needle using a syringe pump (KDS 100, KD Scientific, Holliston, MA) at a flow rate of 1.5 ml h⁻¹ and electrospun at a high voltage (15 kV) to obtain nanofibres of HA incorporated in gelatin (Gel/HA nanofibres). Nanofibres were collected on glass coverslips of 15 mm diameter

placed on a ground aluminium plate for cell culture experiments, and also directly on a flat aluminium plate for chemical and mechanical characterization studies. The electrospun nanofibres were dried overnight under vacuum to remove any residual TFE and moisture. Electrospun scaffolds were subjected to chemical cross-linking by keeping them in desiccators containing 30 ml of 50% glutaraldehyde for 24 h. The cross-linked scaffolds were again dried overnight under vacuum before being used for cell culture studies.

To collect Gel/HA nanofibres by the “spin–spray” method, a rotating cylinder set-up was used instead of a flat collector plate. Aluminium foil was wrapped on the cylinder and 15 mm coverslips were stuck on it using double-sided tape. Gelatin solution was subjected to electrospinning as explained above; at the same time, electrospinning of HA was carried out by feeding a 4% solution of HA in methanol at a rate of 0.5 ml h⁻¹, while the potential between the electrodes was kept at 9 kV. The obtained nanofibrous scaffolds were dried overnight under vacuum and subjected to chemical cross-linking for mechanical characterizations and hFOB cell culture. Fig. 1 shows the schematic diagram of a laboratory set-up used for simultaneous electrospinning and electrospinning.

2.3. Characterization of nanofibrous scaffolds

The electrospun nanofibres were sputter coated with gold (JEOL JFC-1200 Auto Fine Coater, Japan) and the surface morphology of cross-linked and non-cross-linked nanofibrous scaffolds were studied under a field emission scanning electron microscope (FEI-QUANTA 200F, Netherland) at an accelerating voltage of 10 kV. The average diameters of the electrospun fibres were obtained from field emission scanning electron microscopy (FESEM) images using image analysis software (Image J, National Institutes of Health, USA). Moreover, the HA incorporated into the gelatin nanofibres for the fabrication of Gel/HA nanofibres were also observed by transmission electron microscopy (TEM), using a JEOL 2100 microscope at an accelerating voltage of 200 kV.

Attenuated total reflectance–Fourier transform infrared (ATR–FTIR) spectroscopic analysis of electrospun nanofibrous scaffolds was performed on an Avatar 380 spectrophotometer (Thermo Nicolet, Waltham, MA, USA) over a range of 500–4000 cm⁻¹ at a resolution of 2 cm⁻¹, with 64 scans per sample.

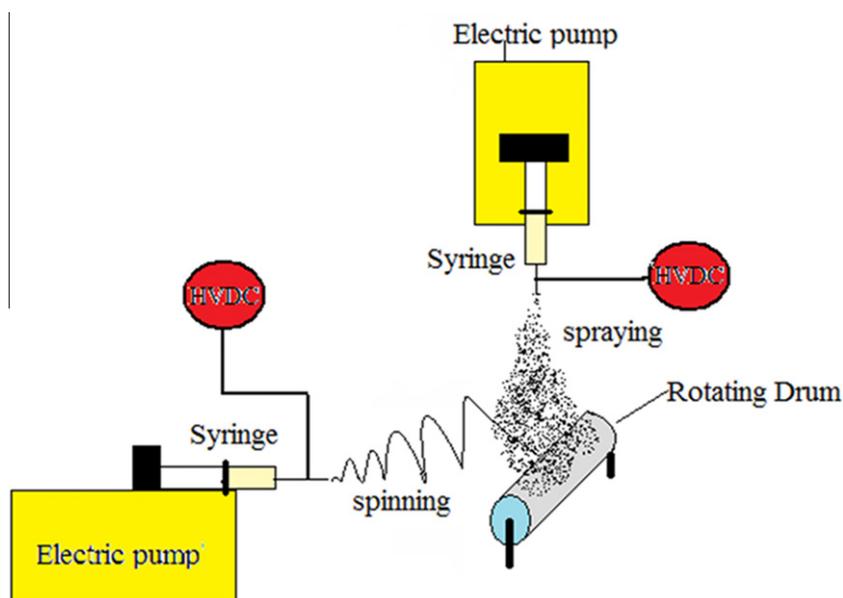


Fig. 1. Laboratory set-up for simultaneous electrospin–electrospraying.

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