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ScienceDirect
Acta Biomaterialia 5 (2009) 2884–2893
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Electrospun nanostructured scaffolds for bone tissue engineering

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Received 29 January 2009; received in revised form 29 April 2009; accepted 7 May 2009
Available online 15 May 2009

Abstract

The current challenge in bone tissue engineering is to fabricate a bioartificial bone graft mimicking the extracellular matrix (ECM) with effective bone mineralization, resulting in the regeneration of fractured or diseased bones. Biocomposite polymeric nanofibers containing nanohydroxyapatite (HA) fabricated by electrospinning could be promising scaffolds for bone tissue engineering. Nanofibrous scaffolds of poly-L-lactide (PLLA, 860 ± 110 nm), PLLA/HA (845 ± 140 nm) and PLLA/collagen/HA (310 ± 125 nm) were fabricated, and the morphology, chemical and mechanical characterization of the nanofibers were evaluated using scanning electron microscopy, Fourier transform infrared spectroscopy and tensile testing, respectively. The in vitro biocompatibility of different nanofibrous scaffolds was also assessed by growing human fetal osteoblasts (hFOB), and investigating the proliferation, alkaline phosphatase activity (ALP) and mineralization of cells on different nanofibrous scaffolds. Osteoblasts were found to adhere and grow actively on PLLA/collagen/HA nanofibers with enhanced mineral deposition of 57% higher than the PLLA/HA nanofibers. The synergistic effect of the presence of an ECM protein, collagen and HA in PLLA/collagen/HA nanofibers provided cell recognition sites together with apatite for cell proliferation and osteoconduction necessary for mineralization and bone formation. The results of our study showed that the biocomposite PLLA/collagen/HA nanofibrous scaffold could be a potential substrate for the proliferation and mineralization of osteoblasts, enhancing bone regeneration.

Keywords: Electrospinning; Bone tissue engineering; Biocomposite nanofibers; Hydroxyapatite; Mineralization

1. Introduction

A major challenge for reconstructive and orthopedic surgery involves the repair of bone defects arising from tumor, trauma or bone diseases. Autogeneic and allogeneic bone functions better in terms of biocompatibility, but requires a second surgery to procure donor bone from the patient's own body. However, with the development of the electrospinning process, electrospun nanofibrous scaffolds with large surface area-to-volume ratio, high porosity, and mechanical properties and morphology similar to the extracellular matrix (ECM) of natural tissue can be fabricated to serve as ideal bone substitutes [1,2]. Non-biodegradable electrospun polymers such as polyurethane and polyesterurethane possess substantial mechanical stability, but might interfere with tissue turnover and remodeling due to their slow degradation [3,4]. Essentially a scaffold needs to maintain its stability and promote cell growth and proliferation, but gradually degrade along with the construction of new tissue and finally to be replaced completely by the new tissue [5]. Various polymeric compositions have been utilized as bone cell supporting matrices by a number of research groups, with the intention of achieving better cellular adhesion, growth and mineral formation suitable for the regeneration of bone. Electrospun polymer/hydroxyapatite (polymer/HA) composites such as polycaprolactone (PCL), collagen and gelatin are being investigated by various research groups for bone tissue engineering [6,7]. Inorganic bioactive materials such as calcium car-

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doi:10.1016/j.actbio.2009.05.007

bonate ($CaCO_3$) were utilized by Fujihara et al. [8] to produce PCL/$CaCO_3$ composites with different weight ratios (75:25, 50:50, 25:75), and these inorganic composites were found to show better alkaline phosphatase expression and osteocalcin. The co-precipitation of HA nanocrystals in soluble collagen has met with partial success in the fabrication of HA–collagen nanocomposites similar to the nanostructure of real bone, though with weaker mechanical properties. On the other hand, Wang et al. produced carbonate-substituted HA–chitosan silk fibroin composites with better compressive strength, mimicking the real bone [9]. The choice of material for scaffold fabrication of tissue-engineered bone needs to meet the essential requirement of providing structural integrity within the body and eventually breaking down, leaving the new tissue which will take over the mechanical load.

Cells attach and organize around fibers with diameters smaller than the diameter of cells [10]. Other than modulating the cellular function to promote tissue regeneration, it is also essential to mimic the unique mechanical properties of bone. The major constituent of natural bone is nanohydroxyapatite (nHA) embedded in collagen matrix [11]. Bioceramic materials such as tricalcium phosphate and HA cannot be easily shaped in the bone defect sites, since they are too brittle. On the other hand, synthetic biodegradable materials are of significance in improving the mechanical properties of such materials, if used together. Poly-L-lactide (PLLA) is a biodegradable polymer, widely utilized as a tissue engineering scaffold, in drug delivery, fixation in orthopedics and as a suitable bio-absorbable membrane [12,5]. Collagen, on the other hand is a natural protein of the ECM and provides better attachment of cells, but gets easily degraded and adsorbed by the body. Bone-like nHA/collagen composites have been developed previously by mineralizing the type I collagen sheet [13,14], though with mechanical properties that are too weak for practical applications. HA, though brittle by itself, has been used for bone regeneration and biomedical implant applications due to its biodegradability, bioactivity and osteoconductive properties [15]. Therefore, biocomposite nanofibers containing HA fabricated by electrospinning might be suitable as a scaffold with improved biological properties for bone tissue engineering. This offers the possibility of fabricating a nano-biomaterial-based scaffold suitable for cellular proliferation with a scaffold strength matching natural bone, and providing a uniform distribution of stresses (load sharing).

We fabricated PLLA, PLLA/HA and PLLA/collagen/HA (PLLA/coll/HA) nanofibers by electrospinning and evaluated the potential of using these substrates for bone tissue regeneration. In the present study, PLLA was blended with collagen and HA, and electrospun to obtain biocomposite PLLA/coll/HA nanofibers that are promising structural scaffolds with suitable cell recognition sites, biocompatibility, osteoconductivity and sufficient mechanical strength for bone tissue engineering.

2. Materials and methods

2.1. Materials

PLLA, molecular weight 100,000 Da, was purchased from Polysciences (Warrington, USA). Collagen type I was purchased from Koka, Japan and HA was kindly provided by the Department of Metallurgical and Materials Engineering, Indian Institute of Technology, Chennai, India. 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) and hexamethyl-disilazane (HMDS) were purchased from Merck, Germany. Human fetal osteoblasts (hFob) cells were obtained from American type culture collection (ATCC, Arlington, VA, USA). Dulbecco's modified Eagle's medium (DMEM), nutrient mixture F-12, fetal bovine serum (FBS), antibiotics, and trypsin–ethylenediaminetetraacetic acid (EDTA) were purchased from GIBCO (Invitrogen, Carlsbad, CA, USA). CellTiter 96 AQueous one solution was purchased from Promega, Madison, WI, USA.

2.2. Electrospinning of nanofibers

The process of electrospinning requires an optimization of various parameters, including the voltage, the distance between the tip of the needle and the collector, as well as the concentration of polymer/blends, solution flow rate, etc., to obtain uniform, random nanofibers [16]. PLLA was dissolved in HFP to obtain a 16% (w/v) solution and was electrospun at a high voltage of 10 kV to obtain PLLA nanofibers. PLLA and HA were mixed at a weight ratio of (80:20) and PLLA, collagen, HA at a weight ratio of (40:40:20) in HFP to obtain PLLA/HA and PLLA/coll/HA nanofibers, respectively. The solutions thus obtained were separately loaded in 5 ml syringes and a high voltage of 12 kV (High Voltage System, Gamma High Voltage Research, FL, USA) was applied. A positively charged jet was formed from the syringe needle and nanofibers were sprayed onto a grounded aluminum plate kept 15 cm away from the tip of the needle. Nanofibers were collected on coverslips 15 mm in diameter for cell culture experiments, while those collected on aluminum foil were used for chemical and mechanical characterization studies. The electrospun nanofibers were dried under vacuum for a week to remove any residual HFP.

2.3. Chemical and mechanical characterization of nanofibers

The electrospun nanofibers were sputter coated with gold (JEOL JFC-1200 Auto Fine Coater, Japan) and visualized by scanning electron microscopy (SEM, FEI-QUANTA 200 F, the Netherlands) at an accelerating voltage of 10 kV. Fiber diameters were measured from the SEM fiber images using Image J software (National Institutes of Health, USA).

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopic analysis of electrospun nanofibrous scaffolds was performed on Avatar 380 FTIR

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