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Enhanced mechanical strength and biocompatibility of electrospun polycaprolactone-gelatin scaffold with surface deposited nano-hydroxyapatite

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ABSTRACT

In this study for the first time, we compared physico-chemical and biological properties of polycaprolactone-gelatin-hydroxyapatite scaffolds of two types: one in which the nano-hydroxyapatite (n-HA) was deposited on the surface of electrospun polycaprolactone-gelatin (PCG) fibers via alternate soaking process (PCG-HA_{AS}) and other in which hydroxyapatite (HA) powders were blended in electrospinning solution of PCG (PCG-HA_B). The microstructure of fibers was examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) which showed n-HA particles on the surface of the PCG-HA_{AS} scaffold and embedded HA particles in the interior of the PCG-HA_B fibers. PCG-HA_{AS} fibers exhibited the better Young's moduli and tensile strength as compared to PCG-HA_B fibers. Biological properties such as cell proliferation, cell attachment and alkaline phosphatase activity (ALP) were determined by growing human osteosarcoma cells (MG-63) over the scaffolds. Cell proliferation and confocal results clearly indicated that the presence of hydroxyapatite on the surface of the PCG-HA_{AS} scaffold promoted better cellular adhesion and proliferation as compared to PCG-HA_B scaffold. ALP activity was also observed better in alternate soaked PCG scaffold as compared to PCG-HA_B scaffold. Mechanical strength and biological properties clearly demonstrate that surface deposited HA scaffold prepared by alternate soaking method may find application in bone tissue engineering.

1. Introduction

The limitations associated with autograft and allograft approach have drawn researcher's interest to develop synthetic grafts to cure bone defects [1–4]. The advancement in nanotechnology has enabled the use of nanofibrous scaffold for bone tissue constructs. Of the various methods available for fabrication of scaffold [5], electrospinning is a simple technique by which fibers of varying dimension from micron to nano range can be prepared by controlling the various parameters such as solution viscosity, voltage, and flow rate [6]. The scaffold material as well as its architecture and topography greatly influence the cellular response of scaffold [7,8]. Electrospun fibers mimic the extracellular (ECM) matrix of bone which provide support to cells and guide cellular behavior [9]. There are various synthetic polymers which have been successfully electrospun into nanofibrous scaffold [10]. To increase the biomimeticity of synthetic polymers, natural polymers such as collagen [11], gelatin [12] and chitosan [13] have been blended with them and composites have the advantages of both, with improved biocompatibility, tunable mechanical properties and degradability. For this study, we have chosen two widely used FDA approved materials; polycaprolactone and gelatin. PCL was selected as the synthetic polymer due to its biocompatibility, stability, better shelf life and low cost [14–16]. Another advantage of PCL is its slow degradation rate in in vivo environment and degradation products do not generate acidic environment in surrounding [17]. To provide the biomimeticity to PCL, gelatin was mixed as a natural polymer because of its well known biocompatibility, biodegradability and low cost in comparison to collagen [18].

Hydroxyapatite (HA), the major inorganic component present in human bone has been widely used as filler material, as coating on bone implants and to functionalize polymeric scaffolds which lack cellular recognition sites. HA coatings improve biocompatibility as well as provide osteophilic surface to implants for bonding with natural bone after implantation [19]. The presence of HA on the surface of polymeric scaffold offers several benefits: it furnishes synthetic polymer scaffolds bioactive and osteoconductive; it changes the chemistry and as well as topography at the surface [20]; HA functionalized scaffold has shown better attachment and proliferation of osteoblasts [21] and it facilitates differentiation of mesenchymal stem cells towards osteoblastic lineage [22]. Budiraharjo et al. fabricated HA coated carboxymethyl chitosan scaffold and observed better attachment, proliferation and differentiation of osteoblasts on HA coated scaffold as compared to non coated scaffold [23]. HA composited scaffolds so far as reported in literature have been mainly produced by three methods: 1 — Blending of HA into polymeric solution [24–29] in which poor dispersion and agglomeration of HA particles occur due

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to low affinity of hydrophilic HA particles towards organic solvent [30,31]. 2 — Soaking in simulated body fluid (SBF) [32–36] which uniformly precipitates HA layer over surface of scaffold but it requires several days for HA deposition [7]. 3 — Alternate soaking method developed by Taguchi et al. [37] which offers several advantages such as being simple, efficient and faster than other methods for HA precipitation [38,39].

Here, we hypothesized that n-HA coated scaffolds resemble more closely to bone environment and enhance the cellular activity towards bone formation as compared to HA blend scaffolds. In the past, various scaffolds with HA blending and soaking have been widely investigated with bone tissue engineering prospects [24,25,40,41]; but scaffolds fabricated from both the methods have never been compared in the same system under identical experimental conditions. In the present study, we have compared physico-chemical and biological properties of two kinds of scaffolds PCG-HA_B and PCG-HA_{AS} fabricated by blending and alternate soaking method respectively. PCG-HA_{AS} scaffold showed sound mechanical properties and biological response as compared to PCG-HA_B scaffold that proves alternate soaking as an efficient method for HA mineralization on scaffold for bone tissue engineering.

2. Experimental section

2.1. Materials

Polycaprolactone (PCL) with average molecular weight 80,000 Da, Gelatin (Type A), 1,1,1,3,3,3-hexafluoro-2-propanol (HFP), trypsin-EDTA, penicillin–streptomycin, Dimethyl sulfoxide (DMSO) and ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS), Dulbecco's phosphate-buffered saline (PBS) and Dulbecco's odified eagle's medium (DMEM) were purchased from Gibco-BRL (Grand Island, NY). Hydroxyapatite powders were a kind gift from Plasma Biotal Ltd., UK. Calcium chloride ($CaCl_2$), di-sodium hydrogen phosphate (Na_2HPO_4) and tris (hydroxymethyl) aminomethane-hydrochloride (Tris–HCl) were purchased from Merck, India. Water was distilled and deionized (DDW) using Milli-Q system (Millipore, MA, USA).

2.2. Preparation of scaffolds

The procedure used for preparation of electrospun scaffold is as follows:

(1) 12% polycaprolactone/gelatin (3:1 ratio; weight by weight) polymer solution was prepared in HFP and kept on stirrer for overnight at room temperature. This ratio was optimized to fabricate scaffolds with sound mechanical properties. The polycaprolactone/gelatin 1:1 and 2:1 ratio exhibited low tensile strength and modulus as compared to 3:1 ratio, therefore this ratio was selected for the further study.
(2) The solution was filled in 5 mL plastic syringe, connected to a blunt end stainless steel hypodermic needle (24 Gauge) (BD, India).
(3) The syringe was fixed to syringe pump (New Era Pump System Inc., USA) and needle tip was connected to the positive output of a high voltage power supply (Gamma High Voltage, USA).
(4) A metallic plate covered with aluminum foil was used as collector and grounded.
(5) The operating parameters for electrospinning were needle tip to collector distance of 12.5 cm, voltage of 14 kV and flow rate of 1.0 mL/h. The entire procedure was carried out in a fume hood at room temperature and at 55% relative humidity. The electrospun scaffold was kept under vacuum for overnight before the mineralization process.

Polycaprolactone/gelatin (PCG) scaffolds were surface functionalized with nano-hydroxyapatite (n-HA) using alternate Ca–P soaking method [42] as described by Taguchi et al. The n-HA coating on PCG scaffold was performed as follows:

(1) PCG scaffold was first immersed in 0.5 M $CaCl_2$ (Merck, India.) solution buffered with 0.05 mol L^{-1}Tris-HCL (pH 7.4) for 30 min at 37 °C.
(2) PCG scaffold was taken out of $CaCl_2$ solution and repeatedly washed with deionized water (DI).
(3) The scaffold was then immersed in 0.3 M Na_2HPO_4 solution buffered with 0.05 mol L^{-1} Tris–HCl (pH 9.0) for 30 min at 37 °C.
(4) PCG scaffold was taken out from the PO_4^{3-} solution and was rinsed with deionized water for several times.

These four steps comprised one soaking cycle. Polycaprolactone-gelatin-hydroxyapatite$_{\text{Alternate soaked}}$ (PCG-HA_{AS}) was prepared by such 2 cycles. After the second cycle, scaffolds were left in DI water for 1 h at 37 °C and then lyophilized for 24 h. This n-HA functionalized PCG scaffold was labeled as PCG-HA_{AS}. All PCG-HA_{AS} scaffold used for characterization and cell culture experiments were prepared after two alternate soaking cycles.

For the preparation of polycaprolactone-gelatin-hydroxyapatite$_{\text{Blend}}$ (PCG-HA_B) scaffold, HA powders (42% by wt.) were weighed, dispersed in HFP, sonicated for 30 min, mixed with the PCG solution, stirred for 6 h and then electrospun with same conditions as used for PCG scaffold fabrication.

2.3. Characterization of scaffolds

The morphological analysis of electrospun PCG, PCG-HA_{AS} and PCG-HA_B scaffolds was done by a scanning electron microscope (Hitachi, S-3400N, UK) at an accelerating voltage of 15 kV. Before this, the samples were cut into 5×5 mm square, mounted on to stub, coated with gold by sputter coating using a SC7640 Sputter Coater (Quorum Technologies Ltd, UK) and observed under SEM (Hitachi, S-3400N, UK). The fiber diameter range of the scaffolds was calculated with image analysis software (ImageJ, National Institutes of Health, Bethesda, USA) from the SEM micrographs. Transmission electron microscopy (TEM, Tecnai 20, Philips FEI, Netherlands) was employed to study the microstructure of fibers at a voltage of 100 kV. Fibers of PCG, PCG-HA_{AS} and PCG-HA_B were collected on TEM grids during the process of electrospinning.

Surface topography of all the scaffolds was characterized using Atomic force microscopy (AFM). AFM studies were performed in tapping mode on PCG, PCG-HA_{AS} and PCG-HA_B scaffolds using Nanoscope IV equipped with 6626E scanner (Digital Instruments, CA). The cantilever used for imaging, is 115–135 nm long probe of silicon nitride, has tip radius of 8–10 nm and 40 N/m spring constant. The scaffolds were cut in a small piece and stuck on metal disk (a puck, 1 cm outer diameter) by double sided cellophane tape.

Thermal gravimetric analysis (TGA) was done to quantify the amount of n-HA present on PCG-HA_{AS} and PCG-HA_B scaffold using NETZSCH-STA 409 PC (Selb, Germany). Samples (n = 3) were loaded within the measurement chamber and heated at a rate of 10 °C min^{-1} up to 750 °C. PCG samples were also analyzed as control.

To assess the mineral phase in PCG-HA_{AS} and PCG-HA_B scaffolds X-ray diffraction was recorded using a PANalytical X'Pert Pro X-ray diffractometer. Cu Kα monochromatic radiation was used with operating parameters of 40 kV voltage and 30 mA current. The XRD patterns were recorded between 20° and 60° (2θ) in step of 0.010 intervals with 1 s counting time at each step.

FTIR spectra were measured in the range of 4000 to 400 cm^{-1} for PCG, PCG-HA_{AS} and PCG-HA_B scaffolds using Nicolet Magna-IR FTIR