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Fabrication and characterization of PCL/gelatin composite nanofibrous scaffold for tissue engineering applications by electrospinning method

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ABSTRACT

In the present study, composite nanofibrous tissue engineering-scaffold consisting of polycaprolactone and gelatin, was fabricated by electrospinning method, using a new cost-effective solvent mixture: chloroform/methanol for polycaprolactone (PCL) and acetic acid for gelatin. The morphology of the nanofibrous scaffold was investigated by using field emission scanning electron microscopy (FE-SEM) which clearly indicates that the morphology of nanofibers was influenced by the weight ratio of PCL to gelatin in the solution. Uniform fibers were produced only when the weight ratio of PCL/gelatin is sufficiently high (10:1). The scaffold was further characterized by Fourier transform infrared (FT-IR) spectroscopy, thermogravimetric (TG) analysis, and X-ray diffraction (XRD). FT-IR and TG analysis indicated some interactions between PCL and gelatin molecules within the scaffold, while XRD results demonstrated crystalline nature of PCL/gelatin composite scaffold. Cytotoxicity effect of scaffold on L929 mouse fibroblast cells was evaluated by MTT assay and cell proliferation on the scaffold was confirmed by DNA quantification. Positive results of MTT assay and DNA quantification L929 mouse fibroblast cells indicated that the scaffold made from the combination of natural polymer (gelatin) and synthetic polymer (PCL) may serve as a good candidate for tissue engineering applications.

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1. Introduction

Tissue loss or organ failure due to severe disease or trauma is a major healthcare problem, as the transplantation of the tissue or organ is not economical and limited by the accessibility of compatible donor [1]. Tissue engineering is a novel scientific approach, which aims to regenerate biological substitutes to repair or replace the damaged organs and lost tissues, and it is based on three important elements: cells, growth factors (signaling molecules) and scaffold (3D polymeric matrix). Scaffold is one of the most important components which interacts with cells and growth factors to regenerate a specific tissue. In our body, the extracellular matrix (ECM) is similar to the scaffold, and consists of 3D nanofibrous structure made of collagen and other biopolymers. Therefore, a 3D scaffold, if made of nanofibers, should provide a biomimetic structure resembling the ECM. The nano-scale feature of a nanofibrous scaffold possesses high surface to volume ratio, which enhances cell adhesion and cell migration, and facilitates nutrient supply to the cells more efficiently [2,3]. For example, the nanofiber architecture has been shown to selectively promote osteoblast proliferation and differentiation in carbon nanotubes [4]. In human corneal epithelial cells, nanogrooved surfaces can induce contact guidance, causing them to elongate and align their cytoskeleton along these topological features [5]. Besides nanofibrous features, properties, such as hydrophilicity, hydrophobicity, mechanical strength and cell-matrix interaction greatly depend on the characteristics of polymer or polymers used for making tissue engineering scaffold. In general, a single polymer cannot impart all the required properties to the scaffold, but by taking a mixture of polymers, it is possible to tailor a composite scaffold with desired characteristics. PCL is a linear, hydrophobic, synthetic polymer [6] that shows high mechanical strength [7]. Although the electrospun PCL nanofibrous scaffold, architecturally mimics the ECM in living tissues, its poor hydrophilicity caused a reduction in its ability of cell adhesion, migration, proliferation, and differentiation [8,9]. On the other hand, gelatin is a biocompatible, biodegradable, and natural biopolymer derived from collagen, a major component of native ECM. It is non-immunogenic and apparently retains informational signals such as the arginine–glycine–aspartic acid (RGD) sequence, which promotes cell adhesion, differentiation and proliferation [10]. Therefore, gelatin can be combined with PCL to obtain a composite scaffold having good cell adhesion and proliferation properties with good mechanical strength. Electrospinning of PCL/gelatin to fabricate PCL/gelatin composite scaffold, has been reported previously [10,11] but solvents used there, were hexafluoro-2-propanol (HFP) [10] and trifluoroethanol (TEF) [6,11] which are very costly and the drastic nature of these solvents may lead to faster degradation of the polymers [12].

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In the present work, our aim is to fabricate a composite scaffold of PCL/gelatin by electrospinning method using a new solvent mixture; chloroform/methanol for PCL and acetic acid for gelatin, and to evaluate the scaffold for its tissue engineering applications. These solvents, chloroform/methanol [13] for PCL and acetic acid [14] for gelatin, are less toxic and cheaper as compared to earlier used solvents (HFP and TEF) [15,16] for PCL/gelatin electrospinning that is why we have chosen these solvents for our study. To the best of our knowledge, we are the first to apply the solvent combinations — chloroform/methanol for PCL and acetic acid for gelatin, for fabricating nanofibrous PCL/gelatin composite scaffold by electrospinning.

2. Materials and methods

2.1. Materials

PCL pellets (Mw = 80,000) and gelatin powder of bovine skin were purchased from Sigma-Aldrich (St. Louis, MO). Glacial acetic acid was procured from Qualigens Fine Chemical, Mumbai, India. Chloroform and methanol were purchased from Fisher Scientific, Mumbai, India. 3-(4, 5-dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide) (MTT), Dulbecco's modified eagle medium (DMEM) and phosphate buffer saline (PBS) were obtained from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum was received from Hyclone, U.S.A., and L929 fibroblast cell lines were received from NCCS, Pune, India.

2.2. Eletrospinning method

The electrospinning was done with the help of an electrospinning setup which is equipped with a syringe having flat-end metallic needle, a syringe pump (Model 11 Plus, Harvard Apparatus) for controlling feed (polymer solution) flow rate, a high voltage DC power supply for charging the polymer solution and a metallic collector or a counter electrode for collecting nanofibers. The solution is taken inside the syringe tube, and a smaller flow rate of the polymer solution is maintained, whereby initially, a droplet produced at the tip of the needle, was held at the tip due to surface tension force. On gradually increasing the voltage, there will be increase in charge–charge repulsion on droplet–surface of the polymer solution. Once the charge–charge repulsion overcomes the surface tension force of the polymer solution, the droplet is elongated while a jet is ejected from the needle-tip, which travels toward the collector (counter electrode; aluminum foil used as collector in this experiment). While traveling to the collector, the jet starts whipping due to bending instability, and elongated gradually, which causes thinning of the jet, and ultimately it is deposited on the collector in the form of nanofibers. The solvent present in the solution-jet evaporates while travelling the distance from the needle-tip to the collector. The details of the mechanism of fiber formation are explained by Greiner and Wendorff [17].

2.3. Preparation of spinning solutions

Firstly, PCL (20 wt.%) has been dissolved in the chloroform/methanol (3:1, v/v) solvent mixture by agitating the mixture with magnetic stirrer at 600 rpm for 2 h at room temperature (26 ± 1 °C). Similarly, gelatin (8 wt.%) was dissolved in acetic acid (80% v/v) by stirring the mixture at 500 rpm for 3 h at room temperature. After the preparation of both polymeric solutions, PCL and gelatin solutions were mixed at different volume ratios (20:80, 40:60, 60:40 and 80:20) with the help of a vortex and kept the mixture for 48 h. After 48 h of incubation, gelatin solution was dispersed in PCL solution whereby an immiscible blend (emulsion) of PCL/gelatin was obtained which was used for electrospinning.

2.4. Fabrication of composite nanofibrous scaffold

For the process of electrospinning, the solution of PCL/gelatin was placed in a 3 mL plastic syringe fitted with a flat needle with a tip diameter of 0.56 mm. The voltage applied was fixed at 22 kV, a feed flow rate of 0.2 mL/h and a distance of 10 cm between needle and collector were maintained throughout the electrospinning process. The nanofibers were collected on a 15×15 cm^2 flat aluminum plate. A syringe pump (model 11 Plus, Harvard apparatus U.S.A.) was used to control the feed flow rate. The nanofiber production experiment was carried out at 26 ± 1 °C (room temperature) and at 45% of humidity.

2.5. Characterization of composite nanofibrous scaffold

The morphology of the composite nanofibrous scaffold was studied with FE-SEM (Quanta 200F Model, FEI, Netherland) at an accelerating voltage of 15 kV. Before imaging, the scaffolds were coated with gold using a sputter coater (Biotech SC005, Switzerland). Fiber diameters of scaffold were calculated on the basis of FE-SEM images at 5000× magnification by using image analysis software (Image J, NIH, USA).

For characterization, XRD, TGA, DTG and FT-IR analyses were performed of fabricated composite scaffold. The experiments were also performed simultaneously by taking components of scaffold i.e., PCL pellets and gelatin powder in order to observe any possible interaction between the molecules of PCL and gelatin in fabricated composite scaffold.

XRD patterns were recorded by X-ray diffractometer (Bucker, AXS D8 Advance diffractometer), using Cu anode at 40 kV and 30 mA in 2θ range of 5–60°.

TG analysis was performed by TGA (EXSTAR, TG/DTA 6300), and each sample (4.0 mg) was run from 22 to 700 °C at a scanning rate of 30 °C/min under a nitrogen atmosphere.

FT-IR spectra of all components were recorded by using thermo nicolet FT-IR (Nexus, USA). The samples were prepared by processing compressed potassium bromide disk and the ratio of samples to KBr used for performing FT-IR analysis was 1 mg samples/900 mg KBr.

To ensure any possible interaction between PCL and gelatin, the dissolution of gelatin was studied by immersing the scaffold in sterile distilled water. Pre-weighted scaffolds were immersed in sterile water at 37 °C and incubated for different time periods (1 and 2 days). After incubation period, the scaffold was dried under vacuum for 24 h. The dried scaffolds were weighed to observe any weight loss.

2.6. Cell viability assay

The viability of L929 mouse fibroblast cells on composite nanofibrous scaffold was quantitatively determined using the colorimetric MTT assay. L929 mouse fibroblast cell line was maintained in DMEM with 10% FBS at 37 °C in a 5% CO_2 incubator (BINDER, Germany). Before cell seeding, scaffolds (0.5×0.5 cm^2) were sterilized with UV radiation from both sides for 30 min each. After sterilization, the scaffolds were incubated with DMEM overnight to make scaffold surface more efficient for cell attachment. Subsequently, the scaffolds were incubated in 96 well tissue culture plate with L929 mouse fibroblast cells at a density of 2×10^5 cell mL^{-1} for 24, 48 and 72 h at 37 °C in 5% CO_2 incubator. After incubation period, the wells were washed with PBS and then 90 μL of fresh complete media and 10 μL of MTT solution (5 mg/mL stock in PBS) were added to the wells to make a final volume of 100 μL. The plate was then incubated at 37 °C for 4 h until purple formazan crystals were formed due to reduction of MTT by viable cells. The media was discarded and 200 μL of DMSO was added to the wells to dissolve the formazan crystals. After removing the scaffold from each well, absorbance was taken in a Biorad plate reader at 490 nm with the subtraction for plate absorbance at 650 nm.

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