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Starch based nanofibrous scaffolds for wound healing applications

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ABSTRACT

Starch is an attractive polymer for wound healing applications because of its wide availability, low cost, biocompatibility, biodegradability and wound-healing property. Here, we have fabricated starch-based nanofibrous scaffolds by electrospinning for wound healing applications. The diameter of the optimized nanofibers was determined by field emission scanning electron microscopy (FE-SEM) and was found to be in the range of 110–300 nm. The mechanical strength (0.5–0.8 MPa) of the nanofibrous scaffolds was attuned using polyvinyl alcohol (plasticizer) and glutaraldehyde (crosslinking agent), to impart them with sufficient durability for skin tissue engineering. Absence of negative interactions between the polymers was confirmed by Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR), differential scanning microscopy (DSC) and thermal gravimetric analysis (TGA). Cellular assays with L929 mouse fibroblast cells indicated the ability of the scaffolds to promote cellular proliferation, without exhibiting any toxic effect to the cells. Thus, the nanofibrous scaffolds demonstrated potential for wound healing applications.

1. Introduction

Tissue engineering is an interdisciplinary biomedical approach that aims to regenerate, reconstruct or repair damaged tissues by supporting the cells with the help of artificial 3D materials [1]. These 3D scaffolds material assist in mimicking the activity of natural extracellular matrix that accommodates the cells in their natural milieu [2]. The cells proliferate and migrate within this matrix to assume their inherent orientation and arrangement. The entire process is governed by the dimension, pore geometry and pore size of the scaffolds, which play a critical role in providing oxygen and nutrient transport to the growing cells. Although various processes have been investigated to develop scaffolds with varying physicochemical properties, the electrospinning technique has proven to be particularly attractive due to its ability to generate scaffolds composed of uniform nanofibers. Moreover, the method offers flexibility to manipulate the fiber dimensions to provide surface area and porosity suitable for diverse biomedical applications. A particularly attractive application is the ability of these nanofibrous scaffolds to simulate the natural extracellular matrix (ECM) and allow adhesion and proliferation of cells seeded on their surface [3]. Besides, the nanofibers provide a surface area to volume ratio and suitable tensile strength to sustain cellular growth. These properties have encouraged increasing employment of this technique in tissue engineering and for fabricating biomedical implants [4]. Despite the simplicity of the process, processing of natural polysaccharides by electrospinning remains a challenging task. The complex chain conformation of polysaccharides and their hydrodynamic responses and repulsive forces, while in solution, negatively influence the spinning efficiency and production of reproducible nanofibers from this class of polymers. In this investigation we have employed electrospinning for producing nanofibrous scaffolds of starch biopolymer. Starch-based biomaterials and it scaffolds have been previously used for several biomedical applications [5]. Starch, as a material by itself, has been explored for wound-healing [6–8]. Also, starch-based scaffolds have been used for adhesion, proliferation, differentiation and regeneration of cells. In recent study, enhanced regeneration of epithelial tissue was

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achieved during wound healing, with the help of collagen, chitosan and starch membrane. Thus, starch-based scaffolds have immense therapeutic significance in wound healing [9]. It has received much attention in drug delivery and other biomedical applications because of its extensive availability, low cost and total composability without generating any hazardous residues. Employment of starch for biomedical functions is also appealing due to its similarity to the native cellular milieu [10]. However certain fundamental properties of starch such as its mechanical properties and moisture sensitivity have to be manipulated to enable its applications in tissue engineering, at par with some commercial expensive polymers [11]. The inherent properties of starch can be altered by with help of polyvinyl alcohol (PVOH), a non-toxic, water soluble, biocompatible, synthetic polymer that can reduce the repulsive forces produced in starch solution and can thus allow electrospinning of its fibers [12]. Another important attribute of starch is its tendency to undergo gelation upon heating, which confers it Non-Newtonian fluid nature. Retrogradation is another characteristic of starch that causes its separation from water, upon cooling, resulting in two different phases [13]. These properties of starch may complicate its electrospinning in the form of aqueous solution, stressing the need to employ a modified solvent system.

In the present work, we report the development of starch-based nanofibrous scaffolds using PVOH as the plasticizer. Electrospun nanofibers of starch-PVOH combinations were successfully developed using hydro-alcoholic solvent for the polymers, for end-application in wound healing. The process was optimized to yield nanofibers with diameters ranging between 110 and 300 nm. The nanofibrous sheet was subsequently cross-linked to enhance its mechanical properties. Evaluation of the optimized nanofibrous scaffolds in cellular assays involving L929 mouse fibroblast cells demonstrated their non-toxicity and their ability to promote cellular proliferation.

2. Materials and methods

2.1. Materials

Potato starch was kindly gifted by Signet Chemical Corporation Pvt. Ltd., Mumbai, India [14]. Absolute ethanol and glutaraldehyde were received from SD fine Chem. Ltd., Mumbai, India. Water soluble PVOH (MW ~ 18,000 g/mol; Degree of hydrolysis = 86.5–89.0 mol %) was purchased from Himedia Laboratories, Mumbai, India. The electrospinning unit, EspinNano-2, was procured from Physics equipment, Chennai, India. Dulbecco's Modified Eagle Medium (DMEM) and EZBlue Cell Assay kit were obtained from Himedia Laboratories, Mumbai, India. The cell line L929 mouse fibroblast was procured from National Centre for Cell Sciences (NCCS), Pune, India. Deionized and double-distilled water (Milli-Q Plus system, Millipore, Bedford, MA, USA) was used in all the experiments.

2.2. Preparation of nanofibers using electrospinning

The electrospinning unit (EspinNano-2, Physics equipment, Chennai, India) was equipped with syringe of 5 mL volume and needles with internal diameter of 0.5 mm. In brief, a blend of starch and PVOH in water was heated up to 70 °C for preparing a homogeneous polymeric solution. Starch to PVOH (S/PVOH) ratio was maintained at 30:70 w/w during the experiments. The spinning dope was prepared at different polymer-blend concentrations of 10%, 12% and 14% w/v using 10% v/v absolute ethanol as the solvent system. Various process parameters were optimized to obtain nanofibers of suitable dimensions. The flow rate was varied between 0.3 mL/h to 0.5 mL/h, while the voltage was varied between 15 and 37 kV. A rotating collector drum covered with aluminum foil was used to collect the ejected fibers, whose distance from the tip of the needle was varied in between 13 and 23 cm.

2.3. Surface morphology

The surface morphology of the nanofibers was studied by Field Emission Scanning Electron Microscopy (UltraPlus Zeiss-4048, Germany). Small pieces of nanofibrous mats were used for the investigation (3 mm × 3 mm). The samples were sputter coated with gold (Quorum Technology, Q150ES, UK) for 30s. The coated samples were mounted over the stubs with the help of carbon tape and analyzed at acceleration voltage of 15 kV, at 0.2 to 0.3 bar, with working distance of 15 cm. The average diameter and standard deviation of fiber was calculated using image analysis software (ImageJ software v1.383, National Institutes of Health, USA). All samples were analyzed in triplicate.

2.4. Crosslinking of nanofibers

The nanofibrous mats were cross-linked to enhance their mechanical property. The prepared mats were compressed on a compression machine (Model MP-15; Technosearch Instruments, Mumbai, India) under a pressure of 10 ton, at room temperature in order to improve their mechanical properties, uniformity and also to minimize the void contents between fibers. Then compressed mats (dimension of 10 mm × 10 mm) were immersed in glutaraldehyde solutions of varying strengths (25%, 15%, 12.5% and 5%v/v; 1 mL) for 12 h. Thereafter, glycine solution (1 mL; 0.02M) was added and kept for 6 h to remove excess glutaraldehyde. The samples were then washed with water, six times, periodically at intervals of 30 min to remove glycine or glutaraldehyde.

2.5. Mechanical strength

The tensile or mechanical strength of the nanofibrous mats was measured using Universal Tensile Machine (UTM) (Tinius Olsen, Model H5KS, USA). Cross-linked scaffolds were cut into sections of dimensions 13.5 cm × 2.5 cm. The resulting scaffolds were mounted between two clamps and stretched at rate of 50 mm/min with an applied load range of 50 N and gauge length of 50 mm. Tensile strength was recorded in triplicate ($n = 3$) at room temperature and average value was calculated.

2.6. Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR)

The presence of functional groups was confirmed by Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR FTIR; Model-Bruker Alpha platinum FT-IR supported with Opus software). The spectra were obtained between wave numbers 400 to 4000 cm^{-1}. Samples of nanofibrous mats were dehydrated by vacuum drying (45 °C) and then placed over the diamond crystal for analysis. 20 scans were recorded for each spectrum. Smoothing was done wherever necessary to reduce the noise, without loss of any peak. The effect of crosslinking was also studied to study any chemical interaction between the polymers or the amide bond formation during crosslinking.

2.7. Differential scanning calorimetry

Analysis of any possible polymer interaction and the thermal transitions was conducted using Differential Scanning Calorimetry (DSC; Perkin Elmer Model-6, USA). A fixed amount of sample (10 mg) was used throughout the analysis. The weighed samples

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