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Fabrication of highly aligned fibrous scaffolds for tissue regeneration by centrifugal spinning technology

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ABSTRACT

Centrifugal spinning (C-Spin) is an emerging technology which uses centrifugal force to produce ultrafine fibers. Being a voltage free technique it can overcome the limitations of electrospinning. Owing to the unique characteristic features such as high surface area to volume ratio, porosity, mechanical strength and fiber alignment, centrifugal spun (C-spun) fibrous mat has a wide range of scope in various biomedical applications. Higher degree of fiber alignment can be effortlessly achieved by the C-Spin process. In order to prove the versatility of C-Spin system with respect to fiber alignment, Polycaprolactone (PCL) and gelatin were spun taking them as model polymers. The morphological analysis revealed that highly aligned ultrafine fibers with smooth surface are achieved by C-Spinning. Hydrophilicity, porosity and mechanical property results confirm that the C-spun mat is more suitable for tissue engineering applications. *In vitro* and *in vivo* experiments proved that the scaffolds are biocompatible and can be efficiently used as a wound dressing material.

1. Introduction

Interest in submicron fibrous mat for tissue engineering applications has increased in recent past because it offers high surface area for cell adhesion and it mimics the fibrillar structure present in native Extra Cellular Matrix (ECM) [1,2]. The porosity present in the mat aids in diffusion of nutrients which results in rapid proliferation of cells [3,4]. The presence of fibers in the form of mat also ensures easier handling during surgical procedure [5]. At present submicron fibers intended for tissue engineering applications are majorly produced by electrospinning process [6–8]. Even though the electrospinning process produces submicron fibers in mat form, the process has some limitations. The limitations of the process are its low productivity, low safety features, and getting controlled fiber morphology in terms of alignment and reproducibility. Moreover the electrospinning process is an ambient driven fiber formation process and a slight change in humidity affects the fiber production and its quality. A new process that can overcome the above limitations is highly desirable for tissue engineering applications' point of view [9–11].

Fibers produced by the usage of centrifugal force have gained momentum in recent past to due to their high production rate and ease of production of fibers with different morphologies. The major process parameters that influence the fiber production and its quality are the concentration of the polymer, selection of solvent and its evaporation rate, speed of rotation of the spinneret and distance of the collector assembly from the spinneret. Fibers of different morphologies can be attained by modifying the spinneret setup and mode of collection of fibers. Aligned fibrous mat can be obtained at ease using the centrifugal spinning setup and can be used for tissue engineering applications, where guided tissue morphogenesis and remodeling are required [12,13].

The alignment in the fibrous matrices mimics the morphology of the ECM. Generally ECM is predominantly composed of collagen which shows typical fibrillar structure with the diameter ranging from nanometers to micrometers in scale. ECM is generally found in the tendons, ligaments, bones and nerves with regular and well defined architecture. It is believed that well defined architecture of native ECM is essential for aiding cell growth and tissue regeneration [14–17]. Hence, a scaffold which mimics the ECM is likely to aid cell proliferation. Literature reports also suggest that the aligned nanofibers can enhance the cell infiltration of endothelial cells and promote wound healing in rat models [18]. Aligned collagen electrospun scaffolds seeded with fibroblast have shown enhanced cell proliferation compared to random scaffolds because of mechanical stretching [19].

Different polymers and their blends have been spun using our in-house built centrifugal spinning setup with desired morphology profile [20,21]. Polymers such as PCL, Polylactic Acid (PLA), and Polyvinylpyrrolidone (PVP) and their blends have been successfully spun in our present setup. Natural protein based polymers are yet to be attempted using centrifugal setup and in the present study the abovementioned setup was used for developing PCL/gel composite nanofiber mat. The

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C-Spun protein fibers were characterized using various analytical techniques and *in-vitro* studies were carried out using fibroblasts and keratinocytes. *In-vivo* animal experiments were also conducted to study the wound healing process with the developed matrices.

2. Experimental

2.1. Materials

Polycaprolactone (PCL) (M_n 70,000–90,000), gelatin (Gel) from bovine skin (Type B, ~225 bloom), and 2,2,2-trifluoroethanol (TFE) were obtained from Sigma. The mouse fibroblast cell line (NIH 3T3) and human keratinocyte cell line (HaCaT) were obtained from National Centre for Cell Science, Pune, Dulbecco's Modified Eagle Medium (DMEM), antibiotic and antimycotic solutions were purchased from Sigma. Fetal bovine serum (FBS) was obtained from Invitrogen Company. All other reagents used in this study were of analytical grade and used without further purification.

2.2. Fabrication of PCL/gelatin scaffolds

Centrifugal spinning was carried out using 15% w/v solutions of PCL/gel in the ratio of 100/0, 70/30, 50/50, and 30/70 using TFE as a solvent. Prior to centrifugal spinning, the solutions were stirred overnight for maintaining solution homogeneity. Prepared solutions were carefully injected into the pot type rotating spinneret head with the help of syringe using the setup. Centrifugal spinning was carefully carried out under a constant speed of 5000 rpm (Fig. 1). The formed ultrafine fibers were collected on an aluminum foil wrapped over a round bottom collector. For cell culture studies, 15 mm cover slip is attached to the round bottom collector by using double sided tape. The collected ultrafine fibrous webs were kept under vacuum to remove the excess solvent.

2.3. Scanning electron microscopic analysis

Surface morphology and cell adhesion over centrifugal spun fibers were observed under a scanning electron microscope (SEM) (S3400NSEM, HITACHI) at an accelerating voltage of 10 kV. Cells seeded on PCL/gel (70:30) ultrafine fibrous web collected over Thermanox® cover slips were fixed with 2% glutaraldehyde. The cells were again re-fixed with 1% osmium tetroxide after washing thrice with 0.1 M sodium cacodylate buffer. The samples were then dehydrated with increasing gradients of acetone–water mixtures followed by treatment with increasing gradients of acetone–hexamethyldisilazane mixtures. The samples were snap frozen with liquid nitrogen and freeze dried in a lyophilizer. Prior to scanning under the SEM, the samples were sputter coated with gold using a fine coater (E 1010, HITACHI).

2.4. Fourier transform infrared (FTIR) spectroscopy analysis

Infrared spectra of C-Spun PCL/gel fibrous mat were obtained using FTIR spectrophotometer (Perkin-Elmer, USA model). Samples of each fibrous mat were ground and mixed thoroughly with potassium bromide at the ratio of 1:5 and the mixture was made into pellet. The IR spectra of the pellets were recorded at the wavelength range of 500 to 4000 cm^{-1} with the resolution of 4 cm^{-1}.

2.5. Thermal analysis

The thermal behavior of C-Spun PCL/gelatin fibrous matrices was examined by Thermogravimetric Analyzer (TGA) (TA Q50, USA) and Differential Scanning Calorimeter (DSC) (DSC 200F3). TGA measurements were conducted over a temperature range of 0 °C to 700 °C at a heating rate of 20 °C/min under nitrogen purge. DSC measurements were carried out under nitrogen atmosphere at the scanning speed of 10 °C/min with the sample weight of 5 mg. The samples were heated from 0 to 250 °C at a heating rate of 10 °C/min.

2.6. Porosity

Porosity determines the mechanical properties, permeability and structural integrity of the scaffolds. Porosity of the centrifugal spun PCL/gelatin fibrous webs was measured according to the procedure reported in the literature and the calculation of porosity of the web is given below [22].

$$\text{Scaffold apparent density}\left(cm^{-3}\right) = \left(\frac{\text{Mass of the scaffold}(g)}{\text{Area of the scaffold}\left(cm^2\right) \times \text{thickness of the scaffold}\left(cm^2\right)}\right) \quad (1)$$

$$\text{Porosity}(\%) = \left(1 - \frac{\text{Scaffold density}\left(gcm^{-3}\right)}{\text{Bulk density of PCL/gel}\left(gcm^{-3}\right)} \times 100\%\right) \quad (2)$$

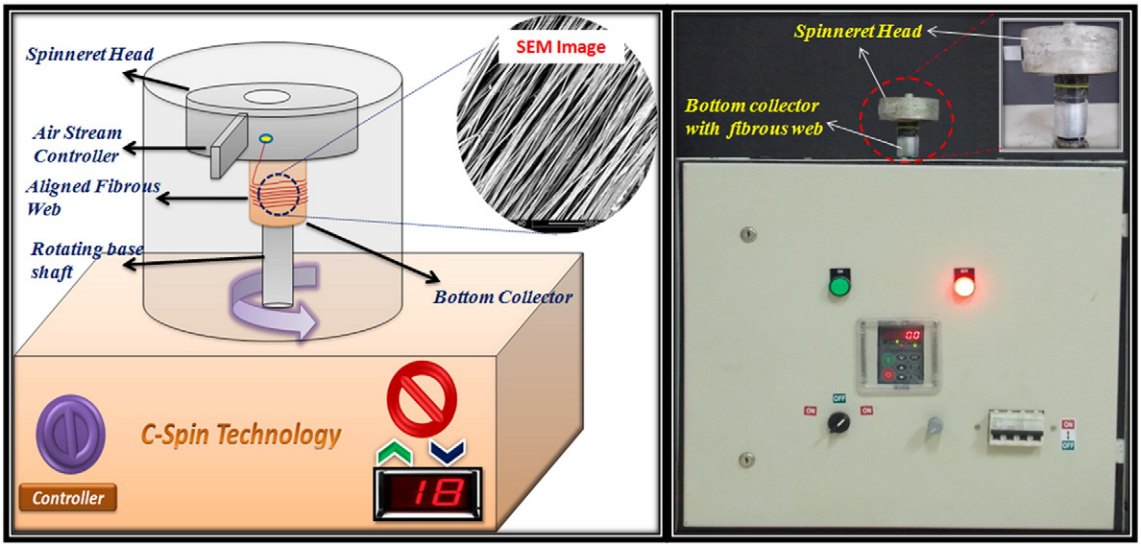

Fig. 1. Prototype centrifugal spinning system.