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Gelatin — Oxidized carboxymethyl cellulose blend based tubular electrospun scaffold for vascular tissue engineering

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ABSTRACT

The present work deals with the fabrication of electrospun tubular scaffold based on in-situ crosslinked blend of gelatin – oxidized carboxymethyl cellulose (OCMC) for vascular tissue engineering. The flow behavior and spinability of the hydrogel despite the in-situ crosslinked gelatin chains evaluated by Raman spectroscopic studies and rheological studies was utilized for electrospinning. The study highlights the tunable pore size and fiber diameter of the nanofibers with the manipulation of electrospinning parameters. With a future perspective of vascular tissue engineering, the electrospinning parameters yielding smooth bead free fibers and maximum magnitude in pore size and fiber diameter as well their homogenous distribution were selected for the fabrication of tubular constructs which is rarely reported. The surface and mechanical properties were evaluated to validate its properties to the native vessel. Biocompatibility was studied *in vitro* with BALB/c 3T3 cells and *in vivo* after subcutaneous implantation in rats. MTT assay confirmed its no-toxicity and no abnormal foreign body reaction were observed by 7 and 15 days after implantation. Crosslinking with biocompatible crosslinker OCMC has rendered insolubility to gelatin yet making it spinable for electrospinning to fabricate porous, nanofibrous vascular biomaterial.

1. Introduction

A scaffold capable of mimicking the extracellular matrix (ECM) of a native blood vessel can prove to be potential for vascular tissue engineering. An attempt to mimic the ECM stimulated the development of a protein based scaffold with nanofibrous morphology. In this aspect, gelatin has the advantage of inherent biological properties of collagen but a greater possibility for modification [1,2]. With the necessity of cell adherence and cell infiltration, additional focus on the architecture of nanofibrous morphology as in the native ECM is required. Electrospinning has emerged as an economic yet effective method for the fabrication of nanofibrous constructs [2,3]. Gelatin has majorly been electrospun in combination with synthetic polymers; mostly by blending or coating. Very few reports show the utilization of electrospun gelatin as the sole matrix [4,5]. The solubility of gelatin and the inadequate mechanical strength limits the use of pristine gelatin. The challenge with crosslinking nanofibers is to retain the nanofibrous morphology and pore dimensions. Hereby, gelatin has been crosslinked by vapors of conventional crosslinkers like glutaraldehyde, formaldehyde *etc*. Zhang et al. crosslinked gelatin nanofibers with glutaraldehyde and observed changed morphology and inhibited cell growth proposed due to residual glutaraldehyde [6]. Natural crosslinkers like genipin, extract from gardenia fruit, has successfully been able to crosslink the nanofibrous structure retaining the morphology and the pore dimensions [7]. However, the extraction of these natural extracts from their sources adds an extra step to the fabrication process elevating the cost of production. An economic way to crosslink gelatin with multialdehyde polysaccharides like oxidized carboxymethyl cellulose, pectin, dextran, chitosan *etc*. has been widely exploited [8–11]. Such gelatin hydrogels have been used as tissue adhesives, drug delivery vehicles and as scaffolds for tissue engineering. Recently electrospun gelatin nanofibrous mat have been crosslinked by immersing in ethanolic solution of oxidized sucrose and borax [12]. Xu et al. has recently fabricated 3D electrospun mesh of zein protein crosslinked by oxidized sucrose which showed higher accessibility and cell penetration rendered due to greater porosity as a potential matrix for tissue engineering [13].

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Jiang et al. evaluated oxidized carboxymethyl cellulose (OCMC) as a crosslinking reagent for carboxymethyl chitosan in comparison to glutaraldehyde as the crosslinker and found that OCMC is a non-cytotoxic, biocompatible and biodegradable crosslinker and resulted in better thermostability and swelling properties of the hydrogel [14].

With our earlier work we had developed a gelatin matrix crosslinked in-situ by partially oxidized carboxymethyl cellulose [15]. An evaluation of the crosslinking parameters and properties of the blend film streamlined that the interfacial interaction between gelatin crosslinked with 20% OCMC facilitated an effectively crosslinked gelatin blend inducing a hydrolytic stability to gelatin. This hydrogel though efficiently crosslinked is spinable due to the randomly spaced, limited aldehyde groups on the partially oxidized polysaccharide. It has improved the usage of pristine gelatin without the use of harmful crosslinkers. The preliminary biocompatibility studies on the films showed that the in-situ crosslinked gelatin blend potentiates to be a matrix for biomedical engineering. However, the lack of interconnected porosity limited its usage as a biomaterial. Since the crosslinking reaction occurs *in-situ* and resulted in a spinable; injectable solution, the matrix could be processed in different ways like film as in our previous work and as electrospun matrix as in the present work. Moreover, electrospinning would provide interconnected porosity and most importantly architecture similar to the nanofibrous native extracellular matrix elevating its potential as a biomaterial. Thence, the present work deals with the fabrication of electrospun tubular construct based on in-situ crosslinked gelatin hydrogel using oxidized carboxymethyl cellulose for the first time as a tubular scaffold as a potential intimal matrix for vascular tissue engineering. An evaluation of the changing properties of the tubular construct namely fiber diameter and pore size with the electrospinning parameters has been carried out. Electrospinning parameters namely, blend concentration in the solvent; applied voltage; feed rate; speed of the mandrel; distance between the tip of the needle and time of spinning have been varied. The optimization of these parameters has been done so as to achieve the maximum as well as homogenous distribution of pore size and fiber diameter. The influence of electrospinning parameters on fiber diameter has been widely reported unlike pore size in this study [16]. Surface and mechanical properties have been estimated. Biocompatibility has been evaluated by *in vitro* MTT assay and *in-vivo* subcutaneous implantation. Nanofibrous morphology with substantial pore size and fiber diameter may enunciate its potential as a matrix for vascular tissue engineering.

2. Materials and methods

2.1. Materials

Gelatin (Type A) from porcine with skin bloom strength of approx. 300 (*i.e.* molecular weight ranging from 50,000 to 1,00,000) was purchased from Sigma Aldrich. Acetic Acid was used as one of the solvents (along with water) procured from Merck (Qualigens). Ultra-pure water, resistivity less than 18 MΩcm, produced by a Millipore Milli-Q system was used throughout the experimental work.

2.2. Preparation of in-situ crosslinked gelatin hydrogel

In-situ crosslinked gelatin hydrogel using oxidized carboxymethylcellulose (OCMC) was prepared according to our earlier publication [15]. Briefly, aqueous solutions of gelatin and OCMC (80:20 composition) were blended at 30 °C for 16 h to obtain in-situ crosslinked gelatin hydrogel in a solvent mixture of acetic acid and water (3:2).

2.3. Characterization of in-situ crosslinked gelatin hydrogel

2.3.1. Raman spectroscopic studies

Gelatin-OCMC hydrogel according to Section 2.2 and accordingly pristine gelatin solution of the same total polymer concentration were prepared. Raman spectroscopic studies with the hydrogel solutions were conducted on Confocal Laser Dispersion Raman Microscope integrated with FTIR from Renishaw (Model—INVIA), UK. Argon ion laser with a wavelength of 514 nm was used for scanning the range from 100 to 3200 cm^{-1}.

2.3.2. Rheological studies

Crosslinked gelatin hydrogel and pristine gelatin solution of the same polymer concentration was used for comparative oscillatory and shear rheological studies conducted on rotational rheometer; MCR 302 Anton Paar, GmBh, Germany using 25 mm parallel plate at 33 °C (reaction temperature). Frequency sweep analysis was performed at 5% strain subjected to frequency scanning from 0.1 to 1000 rad/s at 1 Hz. For the viscosity dependence on shear rate, shear rate (1/s) ranging from 0.01 to 1000 at 33 °C at 5% strain was used.

2.4. Electrospinning of in-situ crosslinked gelatin hydrogel

Electrospinning of the gelatin hydrogel was carried out using an electrospinning machine by E-Spin Nano Pvt. Ltd. The syringe pump (Cole Parmer®) was loaded with a 5 mL syringe containing the gelatin hydrogel solution which in turn reached the needle *via* a silicon tube having an inner diameter of around 2.0 mm. The needle was connected to the positive output of the power supply (Gamma High Voltage Research). The electrospinning with the variation of gelatin-OCMC blend concentration was carried out on aluminum foil covered static collector. The blend concentration that gave smooth fibers was proceeded with for the rest of the electrospinning parameters on a rotating collector. Thereafter, a grounded rotating mandrel thinly coated (approximately 10–30 μm) with 50% aqueous solution of PEG was used as the collector placed horizontally at the required distance from the needle. The electrospinning parameters namely; mandrel speed, feed rate, applied voltage; distance between the tip of the needle and the collector and spinning time had been varied. The nanofibrous construct was easily removed from the mandrel and dried in a vacuum oven at 50 °C for a week. The dried nanofibers were used for further characterization.

2.5. Fiber morphology

The morphology of the gold sputter coated electrospun fibers was observed through scanning electron microscope (SEM) (ZEISS EVO Series Scanning Electron Microscope Model EVO 50). Prior to imaging, a small section of the dried fiber mat on the sample holder was sputter coated with gold by using a Polaron Gold/Silver Sputter Coating unit. SEM was then used to observe the samples at an accelerating voltage of 15 KV. Image J software was used to calculate the fiber diameter and pore size wherein 30 measurements of each were calculated from 10 SEM images.

2.6. Fabrication of tubular gelatin-OCMC scaffolds and characterization

Subsequent to the optimization of the electrospinning parameters based on the maximum count and homogenous distribution of pore size and fiber dimeter each, tubular scaffolds were fabricated on the PEG coated mandrel using the optimized parameters for

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