ELECTROSPUN GELATIN BASED NANOFIBERS CROSS-LINKED BY NATURAL MOLECULES FOR BIOMEDICAL APPLICATIONS

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by

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ABSTRACT

Electrospinning has a unique ability to produce highly porous and interconnected nanofibers with very high surface area to volume ratio. Natural and synthetic polymers can be produced as nanofibers with diameters ranging from tens to thousands of nanometers with tunable properties. The potential of these electrospun nanofibers in human health care applications is promising in many aspects such as tissue/organ regeneration, vehicle to deliver the drugs, wound healing and dressing materials, etc. Natural polymer nanofibers catch the attention of bioengineering fields due to their biocompatibility and non-toxicity. However, fabrication of electrospun natural polymer nanofibers is challenging, due to the lack of appropriate solvents and requirement of external cross-linking agents. The solvents and cross-linking agents being employed for natural polymers are harsh and toxic materials which limit their biological applications. Electrospun gelatin based nanofibers attract attention of biomedical field because of its excellent biocompatibility and structural resemblance with native extracellular matrix. The focus of this thesis is mainly to fabricate electrospun gelatin nanofibers using benign solvent system and non-toxic and natural cross-linkers for biomedical applications and the improvement of their properties by modifying the gelatin nanofibers.

In this work, gelatin nanofibers were fabricated using an innovative cross-linking approach to minimize cytotoxic effects. The solvent system for electrospinning was optimized to keep the acetic acid concentration as minimum as possible. Gelatin was dissolved in water:acetic acid (8:2, v/v) solution and electrospun to form nanofibers with diameters in the range of 150 ± 30 nm. Electrospinning was carried out by varying the amount of gelatin until beadless and smooth fibers were formed at 30 % w/v concentration. In order to improve the water stability, the gelatin nanofibers were cross-linked with a modified polysaccharide, namely, dextran aldehyde. Cross-linking with dextran aldehyde could be achieved without compromising the nanofibrous architecture. Cross-linking was carried out in ethanol medium in presence of minimum quantity of aqueous borax solution due to the very low solubility of dextran aldehyde in ethanol. Dextran aldehyde cross-linked gelatin nanofibers maintained the fibrous morphology in aqueous medium. These mats exhibited improved tensile strength (30 ± 3.47 MPa) and Young's modulus (904 ± 68 MPa) compared to the as spun mats (8.29 ± 0.53 MPa and 394 ± 96 MPa). The cross-linked mats showed gradual degradation behaviour up to four weeks under physiological conditions. The nanofibers were evaluated for cytotoxicity, cell adhesion, viability, morphology and proliferation using L-929 mouse fibroblast cells and MG-63 osteoblast cells. The results confirmed that dextran aldehyde cross-linked gelatin mats are non-cytotoxic towards L-929 and MG-63 cells with good cell adhesion, spreading and proliferation.

The shortcoming associated with cross-linking of gelatin nanofibers using dextran aldehyde is the insolubility of dextran aldehyde in ethanol medium leading to low degree of cross-linking. Hence, a disaccharide namely, sucrose was investigated as another cross-linking agent for gelatin nanofibers. Sucrose was oxidized by periodate oxidation to introduce aldehyde functionality. Oxidized

sucrose (sucrose aldehyde) enabled better cross-linking efficiency, since it is readily soluble in ethanol. Sucrose is cost effective, commercially available in large scale and is potentially biocompatible. Cross-linking of the nanofiber mat with oxidized sucrose was achieved without compromising the nanofibrous architecture. Cross-linked gelatin nanofibers maintained the fibrous morphology even after keeping in contact with aqueous medium. Sucrose aldehyde cross-linked gelatin nanofibers also exhibited improved mechanical properties (tensile strength: 38 ± 5.47 MPa and Young's modulus: 1387 ± 90 MPa) with gradual degradation pattern under physiological conditions. The nanofibrous mats were also evaluated for cytotoxicity and cell viability using L-929 fibroblast cells and MG-63 osteoblast cells. The results confirmed that oxidized sucrose cross-linked gelatin nanofibers are non cytotoxic and promote the growth and proliferation of L-929 and MG-63 cells.

In order to further improve the physico-chemical and biological properties of gelatin based nanofibers, modifications were carried out. The modifications are based on chemical reaction, physical mixing and change in instrumental set-up. A novel nanofibrous mat using amine functionalized gelatin was fabricated. Modified gelatin known as cationized gelatin was found to be soluble in water without forming gel at room temperature unlike gelatin. Hence, electrospinning of cationized gelatin could be carried out using water as the solvent. The water stability of cationized gelatin nanofibers was improved by cross-linking with dextran aldehyde and sucrose aldehyde. The resulting cationized gelatin nanofibers were evaluated for the adhesion and proliferation of L-929 and MG-63 cells. The results demonstrated that the electrospun cationized gelatin nanofibers can be potential scaffold materials for tissue regenerations.

Coaxial electrospinning is an upcoming technology that has emerged from the conventional electrospinning process in order to realize the production of nanofibers of less spinnable materials with potential applications. In the present work, core-shell structured polymer nanofibers of purely natural origin were produced from chitosan (shell) and gelatin (core) by coaxial electrospinning. Highly spinnable gelatin is employed as core material and nanofibers were fabricated with chitosan as shell using aqueous acetic acid as solvent. This method avoided the usage of synthetic polymers as core template for the fabrication of the chitosan nanofibers. For maintaining the biocompatibility and structural integrity of the core-shell nanofibers, cross-linking was carried out using the naturally occurring cross-linking agents, dextran aldehyde and sucrose aldehyde. The biological evaluation of the cross-linked core-shell mats was carried out using L-929 and MG-63 cells. The results showed that the dextran and sucrose aldehyde cross-linked core-shell nanofibers are excellent matrices for cell adhesion and proliferation.

In order to further improve the mechanical and biological performance of gelatin based nanofibers, gelatin was blended with graphene oxide (GO). The present study examined the possibility of incorporation of GO into electrospun gelatin nanofibers *via* co-electrospinning. The interaction between GO and gelatin in the nanofiber structure was established with spectroscopic evidences. The reinforcement in mechanical strength of GO loaded gelatin nanofibers was investigated. The tensile strength was increased from 8.29 ± 0.53 MPa to 21 ± 2.03 MPa after the incorporation of GO. The composite nanofibers were cross-

linked with dextran aldehyde and showed further increase in tensile strength up to 56.4 ± 2.03 MPa. The cross-linked nanofibers were evaluated for cell adhesion and proliferation of L-929 cells. The results indicated that the presence of GO not only acted as reinforcement in mechanical properties, but also encouraged the adhesion and proliferation of L-929 fibroblast cells. GO incorporated gelatin nanofibers were evaluated also for antibacterial activity against gram positive (*S.aureus*) and gram negative (*E.coli*) bacteria. However, the interaction between gelatin and the basal planes of GO rendered the composite nanofibers inactive against bacteria. Hence, antibacterial activity was induced into the composite nanofibers by incorporating a broad spectrum antibiotic, gentamicin. The drug loaded mat exhibited an initial burst release during the first 6 h followed by a gradual release of gentamicin. The mats showed antibacterial property against *S. aureus* and *E. coli.*