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Novel electrospun chitosan/polyvinyl alcohol/zinc oxide nanofibrous mats with antibacterial and antioxidant properties for diabetic wound healing

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ABSTRACT

Non-healing wound is a serious complication of diabetes, associated with extremely slow wound closure, and a high rate of infection, resulting in amputation or losses of limbs, high health care cost and poor quality of patient's life. In the present study, we hypothesized that nanofiber mats composed of a combination of chitosan, polyvinyl alcohol (PVA) and Zinc oxide (ZnO) could be an effective option for faster healing of diabetic wounds due to the wound healing activities of chitosan-PVA nanofibers and antibacterial properties of ZnO. Nanofiber mats of chitosan, PVA and ZnO were synthesized using electrospinning technique. The developed nanofibrous mats were characterized by scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), antibacterial and antioxidant assays as well as *in vivo* wound healing experiments in rabbits. The results revealed that chitosan/PVA/ZnO nanofibrous membranes possessed higher antibacterial potential against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* compared to chitosan/PVA nanofibrous membranes. Moreover, chitosan/PVA/ZnO nanofibrous membranes exhibited higher antioxidant potential compared to chitosan/PVA nanofibrous mats. The *in vivo* wound healing studies showed that chitosan/PVA/ZnO nanofibrous membranes resulted in accelerated wound healing as compared to chitosan/PVA nanofibers. The current study, thus, reveals that chitosan/PVA/ZnO electrospun scaffolds could be effectively helpful in dressings for diabetic wounds.

1. Introduction

Impaired wound healing is a severe a medical problem in type-2 diabetic patients leading to chronic wounds and foot ulcers resulting in increased health care costs and poor quality of life [1]. Diabetic wounds are persistent and painful complications comprised of prolonged chronic inflammation, microbial infection, disturbed growth factors secretion and disrupted angiogenesis [2]. These chronic non-healing wounds result in repeated re-hospitalization [3] and often aggravate the conditions, resulting in amputation of the affected limb or organ [4]. This can occur due to a disturbance in collagen anabolism which is one of the main factors related to wound healing, and poor migration, relocation and propagation of fibroblasts and keratinocytes, contributing to poor development of epithelium [5]. Other factors such as lack of vascularization, excessive secretion of tissue degrading enzymes and bacterial/microbial infections also play significant roles. Effective strategies are required to formulate novel effective wound dressings to facilitate faster regeneration of damaged tissues. Available clinical approaches have not yielded desired results, and, therefore, new dressings with effective wound healing potential are an urgent necessity. The common options available for sustained delivery of therapeutic agents to diabetic wound involve nanoparticles, nanogels, beads, biofilms, bandages and nanofibrous membranes carrying active biomolecules for specific functionalities [6–8]. Nanofiber mats, among these formulations, are considered as one of the best formulations due to their strong mechanical properties, ease of fabrication and effective clinical applicability [9,10]. Thus, the development of novel wound dressings using nanofibrous mats containing therapeutically effective biomolecules is a potent option for effective wound healing in type-2 diabetes patients [11,12].

Nanofiber mats from chitosan, poly vinyl alcohol (PVA) and Zinc oxide (ZnO) can be beneficial for wound dressings as these substances possess their own specific therapeutic properties [13]. Chitosan, a natural polycationic polysaccharide biopolymer with strong antioxidant activity, biocompatibility, biodegradation, antimicrobial potential and has been described as a safe agent in tissue engineering, controlled delivery of pharmaceuticals agents and wound dressings. Chitosan is an effective

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material for faster wound contraction as it possesses the potential to enhance fibroblasts proliferation and macrophage recruitment [14]. During the course of wound healing, chitosan breaks up into *N* acetyl D glucosamine which stimulates enhanced fibroblast spread, collagen formation and improved deposition of hyaluronic acid at the site of wound [15]. Chitosan based electrospun nanofiber mats also have immense potential for diabetic wound care since it resembles extra-cellular matrix (ECM) and can promote cell adhesion, proliferation and subsequent wound closure [16]. Nanoparticles have diverse properties; while some such as CdO are very toxic, there are others that are non-toxic such as ZnO, AgO and TiO_2 and are used in many commercial products including detergents, biosensors, food packaging and antimicrobial agents [17]. ZnO nanoparticles, owning to the antimicrobial property as well as their role in fibroblast proliferation and angiogenesis, are used as an active ingredient in wound dressings [18]. Keeping in the view, the role of inorganic nanoparticles in faster closure of wounds, blending chitosan with ZnO nanoparticles can be beneficial for enhancing microbicidal potential and improved collagen deposition in wound area.

Similarly, PVA is a promising non-toxic biocompatible biopolymer mostly used as surfactant in several nanoformulations as it greatly enhances their mechanical properties [19]. PVA based composites serve as useful scaffolds with improved thermal and chemical stability allowing low protein adsorption efficacy for bioadhesive applications [20]. PVA is also added in chitosan to improve the mechanical, biodegradable and hydrophilic properties of composite nanofiber membranes and help in the formation of uniform nanofibers [21,22]. In addition, incorporation of PVA into chitosan nanofibers enhances viability, proliferation and gene expression of fibroblasts cells and thus, improves the biocompatibility of electrospun membranes [23,24]. Electrospun chitosan nanofibrous scaffolds have already been investigated for the soft tissue regeneration, and their role as an agent in wound healing has already been established [16]. Therefore, we proposed that the electrospun nanofibrous mats of chitosan/PVA/ZnO will be promising candidates for the diabetic wound healing and may be used in future for better wound contraction in clinic. In this study, we fabricated chitosan/PVA/ZnO nanocomposite wound dressings and explored their physicochemical and biological properties. The results of current investigations manifested that chitosan/PVA nanofibrous membranes improves the wounds healing capacity in rabbits induced with diabetes whereas the degree of wound closure was enhanced by incorporation of ZnO nanoparticles in chitosan/PVA mats. Thus, chitosan/PVA/ZnO nanofibrous membranes could be useful dressings for faster healing in type-2 diabetic wounds.

2. Material and methods

2.1. Materials

Chitosan mol. wt. 50,000–190,000 Da, ZnO nanoparticles, methanol, 2,2 Diphenyl 1 picrylhydrazyl (DPPH), alloxan monohydrate and PVA (medium mol. Wt., 85,000–124,000 Da, 99.9% DH) were purchased from Sigma Aldrich. Acetic acid was obtained from Merck. Other materials such as yeast extract, NaCl and peptone were also purchased from Sigma Aldrich. Rabbits used in this study were supplied by National Institute of Health (NIH), Islamabad.

2.2. Preparation of chitosan and PVA blends

Chitosan and PVA solutions were prepared as already described by Wang et al. [20]. Chitosan was initially soaked into acetic acid to facilitate its dissolution and later, it was stirred at 50 °C for 2 h to get a chitosan solution. Meanwhile, distilled water was used to dissolve PVA and complete mixing was obtained through condensation process. Chitosan/PVA blend was prepared at a ratio of 1:4 by pouring PVA solution drop wise in chitosan solution. The chitosan/PVA blend was divided in two parts and ZnO nanoparticles size of 40.00 nm were mixed in one of chitosan/PVA part to get chitosan/PVA/ZnO blend while the other blend was used as a blank.

2.3. Electrospinning chitosan/PVA and chitosan/PVA/ZnO blends

The electrospinning setup was used for fabrication of nanofibers as reported by Hasan et al. [25]. In short, mixed blends of chitosan as prepared already were filled into a 10 ml plastic syringe 21-gauge needle. One electrode of the power supply of electrospinning set up was connected to the needle and the other electrode was attached with collector plate and a 7.00-cm distance was maintained between needle and collector plate. Electrospinning process was performed at a rate of 0.5 ml/h and nanofibrous mats were gathered from collector plate. Later, prepared nanofibrous membranes were used for characterization and wound healing experiments on rabbits.

2.4. Characterization of electrospun nanofibers

2.4.1. SEM analysis

Scanning electron microscope (SEM) was used to obtain size, morphology and possible structure of already fabricated nanofibrous mats. First, nanofibrous mats of blank chitosan and chitosan impregnated with ZnO nanoparticles were sliced into 3-mm length and 0.5-mm width sections using a sharp scissor and mounted later a stub with adhesive tape. The gold coating of sections of nanofibrous membranes was carried out in a sputtered in a unit (Edwards, UK). Images of chitosan membranes were taken at 15 kV and later, ImageJ was used in order to get average diameter of nanofibers. The random positions of nanofibers were selected, and average of these measurements gave us the diameter of fibers.

2.4.2. FTIR analysis

The infrared spectrum of the samples comprising chitosan, PVA, ZnO nanoparticles and chitosan/PVA ZnO nanofibers was measured at 4000 and 400 cm^{-1} wavelengths at a 2 cm^{-1}resolution and averaged over 200 scans by using FTIR spectrometer. The measurements of functional groups on the surface of chitosan and chitosan nanofibers incorporated with ZnO nanoparticles were obtained by infrared spectroscopy through transmission mode.

2.4.3. XRD analysis

The structural properties of both chitosan/PVA and chitosan/PVA/ZnO nanofibrous mats were determined by X-ray diffraction. XRD measurements were performed to investigate the integration of the ZnO nanoparticles into the nanofibrous membranes. PANalytical, Netherlands diffractometer was used, and measurements were recorded at continuous scans of 2θ, 5–90° range.

2.5. Antioxidant assays

DPPH assay was used to assess the percentage scavenging potency of ascorbic acid (control), ZnO nanoparticles, CS/PVA and CS/PVA/ZnO nanofibers according to the protocol followed by Hatano et al. [26]. The samples of nanofibrous membranes were dissolved into the methanol and DPPH solution. The samples were mixed vigorously and kept at room temperature for a time of 30 min in dark to allow the completion of reaction. The spectrophotometer was later used, and absorbance of samples was recorded at 517 nm wavelength. The percentage antioxidant activity of each of sample was calculated according to equation used by Hatano et al. [26].

2.6. Antimicrobial activity studies

Study of the antimicrobial activities of nanofibrous membranes were assessed by disc diffusion method (DDM) and colony count method