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CHEMICAL CROSS-LINKING OF CHITOSAN/POLYVINYL ALCOHOL ELECTROSPUN NANOFIBERS

KEMIJSKO ZAMREŽENJE ELEKTRO SPREDENIH NANOVLAKEN IZ HITOSAN/POLIVINIL ALKOHOLA

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Electrospun nanofibrous scaffolds have great potential for many biomedical applications. In the present study, we fabricated and characterized chitosan/polyvinyl alcohol (Chi/PVA) nanofibrous scaffolds through electrospinning. Cross-linking was performed using chemically with 5 % glutaraldehyde vapor. The morphology and chemical banding of the electrospun nanofibers before and after cross-linking were evaluated using scanning electron microscopy (SEM) and Attenuated Total Reflectance-Fourier Transform InfraRed (ATR-FTIR) spectroscopy. SEM micrographs and FTIR spectra showed that the cross-linking process was accomplished successfully. With the biocompatibility and non-toxicity of chitosan and PVA, it is expected that this electrospun nanofibrous scaffold could be an excellent candidate for biomedical applications.

Keywords: electrospinning, chitosan, polyvinyl alcohol, cross-linking

Mreže iz elektro spredenih nanovlaken imajo velik potencial za uporabo v biomedicini. V študiji smo izdelali in karakterizirali nanovlaknasto mrežo, izdelano z elektro predenjem nanovlaken iz hitosan/polivinil alkohola (Chi/PVA). Zamreženje je bilo izdelano s pomočjo kemijske metode s 5 % glutaraldehidne pare. Morfologija in kemijsko povezovanje elektro predenih nanovlaken, pred in po zamreženju, sta bila ocenjena z uporabo vrstičnega elektronskega mikroskopa (SEM) in z metodo z oslabljenim odbojem infrardeče spektroskopije s Fourierjevo transformacijo (ATR-FTIR). SEM-posnetki in FTIR-spekter sta pokazala, da je bil postopek zamreženja uspešno dosežen. Glede na biokompatibilnost in netoksičnost hitosana in PVA se pričakuje, da bodo mreže iz elektro spredenih nanovlaken odličen element za uporabo v biomedicini.

Ključne besede: elektro-predenje, hitosan, polivinil alkohol, zamreženje

1 INTRODUCTION

Electrospinning is a simple, versatile and cost effective method for forming non-woven fibrous scaffolds. Technically, the electrospinning process uses a high voltage source to draw a polymer fluid into fine fibers which are deposited on a collector.[1] In recent years, the use of electrospun nanofibers for biomedical applications such as tissue engineering[2], wound dressing[3], protein immobilization[4], materials for artificial blood vessels[5], barriers for the prevention of induced adhesion after operation[6], and vehicles for drug or gene delivery[7] has attracted a great deal of attention from scientists. Electrospinning of synthetic and natural polymers has been reported for collagen[8], gelatin[9], silk fibroin[10], polyglycolide (PGA)[11], polylactide (PLA)[12] and poly(ε-caprolactone) (PCL)[13], polyurethane[14], poly(vinylalcohol)[15], PEO[16], polydioxanone[17], and polyphosphazene derivatives.[18] Furthermore, the blending of two or more polymers and copolymerization are effective methods for the preparation of composites with new and desirable properties. Obviously, by adjusting the ratio of the components, structure and morphology of the nanofibers and the biological properties of the electrospun scaffolds can be tailored to the desired traits and functions.[1] For example PLGA[7], P(LA-CL) copolymers,[19] and mixtures of collagen with elastin,[20] gelatin with PCL,[9] chitosan with poly(ethylene oxide) (PEO)[21] and chitosan with PVA[22] have all been utilized to fabricate electrospun nanofibrous scaffolds for biomedical applications.

In biomedical applications, after electrospinning, different cross-linking methods can be uses to provide stabilization against aqueous environments for those scaffolds produced from aqueous soluble polymers (For example: PVA).

In the present study, electrospinning of a chitosan and PVA blend was performed. Chitosan was selected due to its cytocompatibility, biocompatibility, biodegradability and antibacterial activity.[23] PVA was used due to its biocompatibility, biodegradability, non-toxicity, chemical resistance, and good fiber-forming properties.[24]

2 MATERIALS AND METHODS

2.1 Materials

PVA (average molecular weight of 70000–100000 g/mol) and chitosan (medium molecular weight) were purchased from Sigma-Aldrich (St. Louis, MO). Acetic

acid and glutaraldehyde were obtained from Merck (Germany).

2.2 Preparation of the solutions

Chitosan and PVA were dissolved in 50 % aqueous solution of acetic acid at a concentration of 2 % mass fraction and 15 % mass fraction, respectively. The chitosan solution and PVA solution were mixed together with a weight ratio of 40/60 (Chi/PVA) under magnetic stirring at 60 °C.

2.3 Preparation of nanofibrous membranes

The optimal conditions for the electrospinning were as follows: 25 kV applied voltage, 15 cm tip-to-collector distance, and 1 ml/h flow rate. Moreover, a 5 ml syringe with a 21 gauge stainless-steel needle was used for the delivery of the polymer solution via a syringe pump.

2.4 Crosslinking of nanofibrous membranes

Samples were exposed to the 5 % glutaraldehyde (GA) vapor at room temperature for 48 h for cross-linking to stabilize them against aqueous media solubility and enhance their biomechanical properties biomedical applications. After crosslinking, the samples were carefully washed several times with 2 % glycine for the inactivation and removal of the GA.[25]

2.5 Characterization of nanofibrous membranes

The morphology and microstructure of the electrospun nanofibers before and after cross-linking were determined by Scanning Electron Microscopy (SEM). The average diameter of fibers were calculated using the ImageJ (US National Institute of Health, Bethesda, MD) image analysis program by analyzing at least 50 fibers in ten SEM micrographs. The chemical structures of the chitosan and PVA powders and Chi/PVA nanofiber membranes before and after cross-linking were investigated by Attenuated Total Reflectance-Fourier Transform InfraRed (ATR-FTIR) spectroscopy (Bruker Tensor 27, USA). FTIR spectra were obtained in the 4000 cm^{-1} to 400 cm^{-1} wavenumber range, with the data analyzed using OPUS software.

RESULTS AND DISCUSSION

3.1 Morphology of the nanofibrous scaffold

SEM images of Chi/PVA nanofibers before and after GA cross-linking are shown in **Figure 1**. As seen in **Figure 1a**, relatively fine, continuous, uniform fiber-structures (no bead), and randomly oriented fibers were obtained. The average fiber diameter was found to be 180±2.28 nm. In accordance with the relatively fine fibers fabricated, it is expected that a suitable porosity exists for biomedical applications.

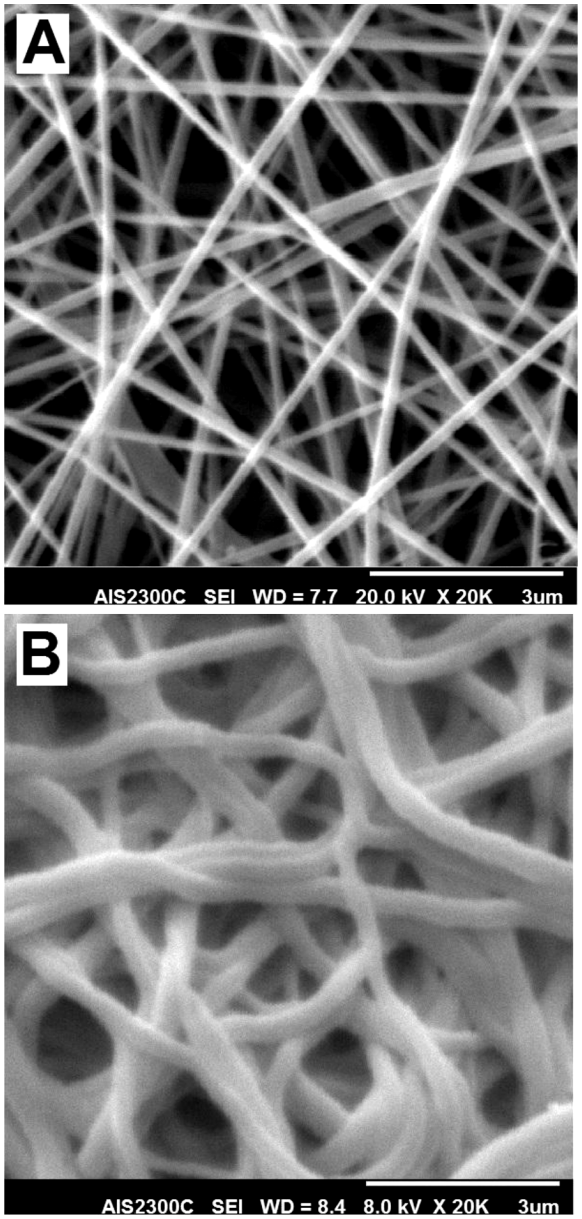

Figure 1: SEM micrographs of electrospun Chi/PVA nanofibers, after 48 h immersion in water at 37 °C: a) before GA cross-linking and b) after GA cross-linking

Slika 1: SEM posnetek elektro spredenih Chi/PVA nanovlaken, po 48 h namakanja v vodi s 37 °C: a) pred GA zamreženjem in b) po GA zamreženju

3.2 Crosslinking of nanofibrous scaffold

The SEM micrographs of the cross-linked nanofibers after immersion in water (at least 48 h) are shown in **Figure 1b**. As PVA is a water-soluble polymer, cross-linking should be performed for the use of Chi/PVA nanofibers in biomedical applications. Several studies have been reported on GA cross-linking for medical application. For example, Jafari et al. used a saturated vapor of a 25 % GA aqueous solution for cross-linking of chitosan-gelatin electrospun nanofibers.[26] In another study cross-linking of electrospun water-soluble carbo-

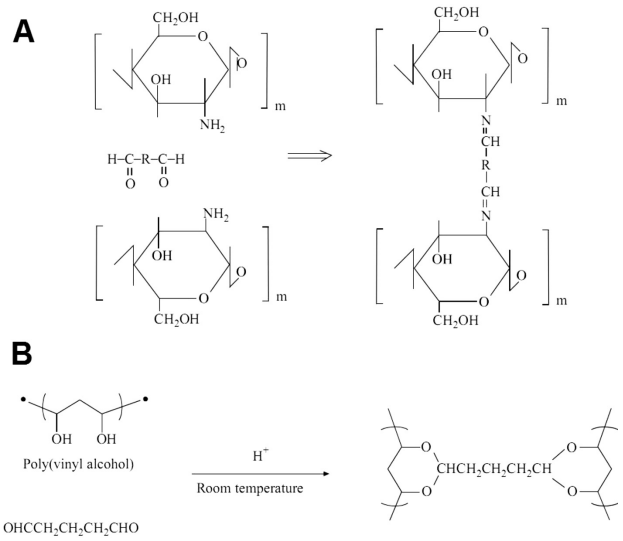

Figure 2: Schematic representation of cross-linking reaction of chitosan with GA and cross-linking reaction of PVA with GA [1,2]
Slika 2: Shematski prikaz reakcije zamreženja hitosana z GA in reakcije zamreženja PVA z GA[1,2]

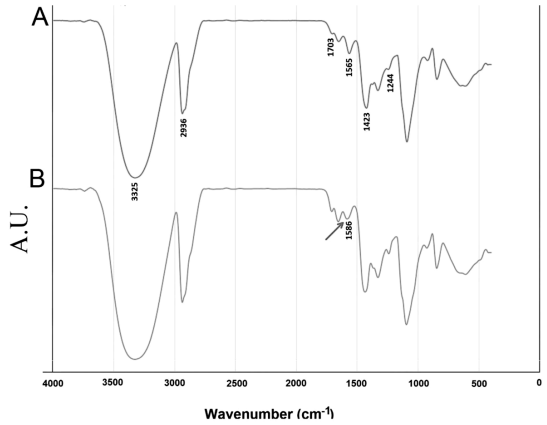

Figure 3: ATR-FTIR analysis of Chi/PVA electrospun samples: a) before chemical electrospinning and b) after electrospinning
Slika 3: ATR-FTIR analiza elektropredenih Chi/PVA vzorcev: a) pred kemijskim elektropredenjem in b) po elektropredenju

xyethyl chitosan/poly (vinyl alcohol) nanofibrous membrane towards wound dressings for skin regeneration was performed using a GA vapor.[27] Unlike the previous work, in the present study, the cross-linking was performed using a lower concentration of GA vapor (5 %). Despite using this low concentration, it was proved that the fabricated membranes' structure is stable in an aqueous solution. Moreover, according to the SEM micrographs shown in **Figure 1**, the porous structure of the fabricated membranes remained intact implying that they are insoluble in water. The cross-linking mechanism of chitosan and PVA with GA is shown in **Figure 2**.[28,29]

3.3 ATR-FTIR analysis

FTIR spectra were taken of the electrospun nanofibers before and after cross-linking, to assess their chemical groups. The FTIR spectrum of the Chi/PVA blended nanofibers before cross-linking, is shown in **Figure 3**. The two peaks at 1423 cm^{-1} and 1565 cm^{-1} arise fromcarboxylic acid and symmetric deformation of $^{-}NH3^{+}$ groups due to ionization of primary amino groups in the acidic medium, respectively. The peak at 1703 cm^{-1} is attributed to the carboxylic acid dimer.[22] In this study, this peak is due to the acetic acid utilized for dissolving the chitosan. The peak located at 1244 cm^{-1} is related to the C–O of the CH2OH chitosan group forming a hydrogen bond with the OH of PVA, confirming the fabrication of Chi/PVA blend nanofibers.[30] The FTIR spectra of the Chi/PVA blended nanofibers before cross-linking is given in **Figure 3**. Chemical crosslinking of the chitosan/PVA is verified by the peak located at 1586 cm^{-1} attributed to the C-N band. All chitosan-derived blends cross-linked with GA, have shown the presence of the imine (C=N) band. The imine band was formed by the nucleophilic reaction of the amine from chitosan with the aldehyde group of GA.[31] Due to imine band instability with temperature and pH, this group can transform to a C-N group.[32]

4 CONCLUSION

In this work, nanofibrous Chi/PVA was fabricated via electrospinning and stabilized by chemical cross-linking using 5 % GA. The porous structure of the electrospun scaffolds, antimicrobial properties of the chitosan and chemical resistant traits of PVA, make our fabricated electrospun scaffold an excellent candidate for biomedical applications. However, *in vitro* and *in vivo* experiments for evaluation of the biocompatibility of these Chi/PVA nanofiberous membranes is necessary.

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