Fabrication of Poly (vinyl alcohol) / Chitosan Nanoweb loaded Ampicillin for Antibacterial Purpose

Neeranut Kuanchertchoo a*, Chayonan Chathathum a, Worapot Chaoon a, Nuanchawee Wetprasit b
Wantana Mongkolvisut c

(a) Department of Materials Technology, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand.
(b) Department of Biotechnology, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand.
(c) Department of Chemistry, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok Thailand.

ABSTRACT

Poly (vinyl alcohol) (PVA) and chitosan were formed into electrospun nanoweb for wound healing and antibacterial purpose. Nanoweb was prepared from 10 wt% of PVA and 9 wt% of chitosan solution with volume ratio of 20:1. Ampicillin sodium salt was added and homogeneously mixed into PVA / chitosan solution before electrospinning. The process parameters such as applied voltage, working distance, and duration for crosslinking with glutaraldehyde vapor were studied. The nanoweb was characterized using SEM, and FTIR. The antibacterial activity was also investigated using disk diffusion method. The results showed that nanoweb was composed of nanofibre with diameter 0.2-0.3 μm. The obtained products significantly inhibited growth of Staphylococcus aureus and Acinetobacter lwoffii. PVA / chitosan nanoweb loaded with ampicillin was expected to be used as wound dressing.

Key words: PVA/chitosan nanoweb, Ampicillin, Antibacteria, wound dressing

Introduction

Poly (vinyl alcohol) (PVA) and Chitosan nanofibers have been prepared and studies their properties as quicker wound healing and new tissue generation (Sandaramurthi, et al., (2012), Chellamani, et al., (2012), and Wang, et al., (2017)). The nanofiber mat from electrospinning process is crucial for wound healing due to govern high porosity and very large surface area to volume ratio. The essential parameters required for wound dressing to heal wounds are absorptivity, oxygen permeability as well as antibacterial properties (Chellamani, et al., (2012)). PVA-chitosan nanofiber mats are expected to be used as wound dressing for chronic dermal wound, i.e., infected diabetic foot ulcers, burns, etc. In the past, Liu et al., (2010) prepared poly (ε-caprolactam) nanofiber yarn loaded ampicillin with high potential for antimicrobial against both gram positive Staphylococcus aureus and gram negative Klebsiella pneumoniae. Pouravari et al., (2016) used electrospinning technique to form PVA / chitosan nanofiber. The nanofiber was then determined properties using SEM and FTIR. Kuanchertchoo et al., (2016) prepared nanofiber mats loaded 1-3 wt% of ampicillin which significantly inhibited growth of Streptococcus agalactiae and Acinetobacter lwoffii. In this research work, PVA / chitosan mixture was electrospun into nanoweb loaded with ampicillin and examined both physical and antibacterial properties.

Experimental

Materials

Poly (vinyl alcohol) purchased from Acros Organics, USA. Chitosan oligomer from crab’s shell with molecular weight 6x10^4-8x10^4 was bought from Mahachai Karnka Co. Ltd, Samutsakorn, Thailand.

*neeranutk@gmail.com
Glutaraldehyde II 25% in water received from Sigma Aldrich Co. Ltd. Tween 20 (polyoxyethylene sorbitan monolaurate) was supplied by Scharlau S.L., Spain.

**Equipment**

Electrospinning unit was setted up with EMCO high volt, negative output voltage 0-300,000 volts and output current 0.33 mA as show in fig 1. FTIR spectrophotometer, model tracer 100 from Shimadzu (Thailand) Co. Ltd., was used to study the functional groups of chitosan. SEM, JEOL (JSM S410LV), was applied to observe the structure of chitosan nanofibres.

![Electrospinning unit](image)

*Homaegohar, et al., 2014, pp. 1020*

**Figure 1. Electrospinning unit**

**Methods**

**Preparation of solutions**

Chitosan (9 wt%) was dissolved in 2 %w/v of acetic acid. Poly (vinyl) alcohol (PVA), (10 wt%) was liquefied in water. The PVA and chitosan solution were then mixed in weight ratio of 20:1. Tween 20 (2 wt%) was then added into PVA/chitosan mixture and kept under constant stirring to obtained homogeneous solution. The ampicillin was loaded at 1, 2, and 3 wt% based on the weight of PVA and chitosan powder and stirred to get uniform composition.

**Electrospinning**

PVA/chitosan solution with different concentrations of and tween 20 were inserted into syringes with needle 0.413 mm inner diameter (No. 22). The filled syringes were setted to the electrospinning system. The needle and collectors were connected to high volt generator cathode and anode respectively. Flow rate of PVA/chitosan solution was controlled to 0.33 ml/min. The electrospinning parameters such as distance between tips to collector (working distance), and applied voltage were studied and discussed.

**PVA/chitosan nanoweb preparation**

PVA/chitosan solution was electrospun and collected by drum for 5 h. The obtained nanowebs were cross linked with glutaraldehyde vapour for 10, 20 and 30 h and kept at 40°C in vacuum oven for 24 h. The properties of nanoweb were examined.

**Characterization of PVA/chitosan nanoweb**

*Fiber diameter*
One hundred fibres from each sample were randomly selected from SEM images and measured using SEMAfore 5 software.

**Attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR)**

The functional groups of the nanoweb was characterized using ATR-FTIR (Perkin Elmer, spectrum 100). Each spectrum was acquired in transmittance mode on ZnSe ATR crystal cell by accumulation of 16 scans with a resolution of 4 cm$^{-1}$ and the wave number of 4000-600 cm$^{-1}$.

**Antibacterial test**

Antibacterial properties of PVA nanoweb containing ampicillin were determined by the zone of inhibition method against two types of bacteria, i.e., the gram negative, *A. lwofii* (ATCC 15309) and gram positive, *S. aureus* (ATCC 25923). The PVA / Chitosan nanowebes were cut into disk shape with diameter 1 cm and placed onto Müller-Hinton agar plate spread with bacteria (10$^5$ CFU/ml). The plate was incubated at 37°C for 24 h, and the inhibition zone or clear zone was measured. The disks without ampicillin addition were used as control samples. The results showed that no clear zone was observed.

**Results and discussion**

**Cross-linking with glutaraldehyde**

After forming into nanoweb, cross-linking process leads to the formation of permanent covalent network, which may not allow the free diffusion of water / bioactive materials and also enhance the mechanical properties (Mi, *et al.*., 2001). The mechanism of PVA or chitosan cross-linked by glutaraldehyde was shown in figure 2 b). PVA and chitosan also have intermolecular interactions as shown in fig 2 a).

![Cross-linking mechanism](image1.png)

**Milosavljevic *et al.*, 2010**

**Pouranvari *et al.*, 2016**

Figure 1 Cross-linking mechanism of a) interaction between PVA and chitosan b) Reaction of PVA or chitosan with Glutaraldehyde.

**Fiber morphology and diameter**

Process parameters in electrospinning, i.e., applied voltage and working distance affected the formation of nanofiber. For higher voltages or field strength, a drop of typically suspended at needle tip, and a jet will originate from a Taylor cone producing bead free spinning assuming that the force of the electric field...
is sufficient to overcome the surface tension. (Deitzel, et al., 2001). Nanofiber was generated with no or little beads as shown in Fig 2 c), f) and i). While a minimum of working distance is required to allow the sufficient time for fibrous to dry before reaching the collector (Geng, et al., 2005). The optimum conditions for forming PVA/chitosan nanofibers were applied electric field of 20 KV with using working distance of 20 cm. The morphology of nanofibers from these conditions was shown in fig 2 e). The diameters of nanofiber of PVA / chitosan with 1, 2 and 3 wt% of ampicillin were shown as fig 3.

Figure 2 Nanofibers were prepared from different working distance and applied voltage a) 15 KV and 15 cm b) 15 KV and 20 cm c) 15 KV and 25 cm d) 20 KV and 15 cm e) 20 KV and 20 cm f) 20 KV and 25 cm g) 25 KV and 15 cm h) 25 KV and 20 cm and i) 25 KV and 25 cm
Figure 3 Nanofibers were prepared from PVA / chitosan with 1, 2 and 3 wt% ampicillin and electrospun using electric field of 20 KV and working distance 20 cm.

**Functional groups of PVA / chitosan nanoweb**

For ampicillin, the sharp and intense peak at 3,385.90 cm\(^{-1}\) was attributed to the N-H stretching. The large band about 2961.76 cm\(^{-1}\) indicates the presence of weak H bonds. The spectral region between 1,800 and 1,600 cm\(^{-1}\), the vibration modes due to C=O group stretching occur at 1,748.67 cm\(^{-1}\) as broader band. The region 1,650-1,500 cm\(^{-1}\) can be observed the \(\nu_{as}\) coo- and \(\delta\) NH\(_3\) bands of zwitterionic form (Baraldi, et al., 2014). While the FTIR spectra of electrospun nanoweb shown in fig 4. PVA nanowebs have the absorption peaks at 3,310, 2916, 1421, 1094 and 850 cm\(^{-1}\) attributed to \(\nu\) (OH), \(\nu\) (CH\(_2\)), \(\delta\) (H-O-H), \(\nu\) (C-O) and \(\nu\) (C-C) respectively (Santos, et al., 2014). While PVA / chitosan nanowebs exhibited the same peaks as PVA nanowebs because of the low amount of chitosan in PVA matrix. PVA / chitosan nanowebs also have no spectra of ampicillin due to low amount added.

![FTIR spectra of PVA/chitosan nanowebs loaded with ampicillin](image)

Figure 4 FTIR spectra of PVA/chitosan nanowebs loaded with ampicillin 0, 1, 2 and 3 wt%. The nanowebs were formed using electric field of 20 KV, working distance 20 cm and cross-linked with glutaraldehyde vapour for 10 min.

**Antibacterial properties**

Ampicillin is an antibiotic with high selectivity which inhibits bacteria cell wall synthesis by binding to specific penicillin-binding protein (PBPs) loaded inside the bacteria cell wall (Liu et al., 2010). PVA / chitosan nanoweb with 1-3 wt% of ampicillin show the antibacterial against S. aureus and A. loeii. The more amount of...
ampicillin loaded into nanoweb, the larger size of clear zone was observed as show in fig 5. The results show that S. aureus is more sensitive to ampicillin than A. lowffii.

![Figure 5 Effect of amount of ampicillin loaded PVA / chitosan nanoweb against a) S. aureus b) A. lowffii](image)

**Conclusions**

PVA/chitosan nanowebs loaded ampicillin were successfully prepared by electrospinning technique using 20 KV, working distance 20 cm and collection time 5 h. For electrospinning parameters, applied voltage affects fiber morphology more than working distance. Fiber diameters were varied from 0.2 -0.3 μm. Crosslinking time should be compromised to get the suitable nanoweb which can be used as antibacterial carrier. PVA/chitosan nanowebs with ampicillin show the same functional groups as those of PVA nanoweb. All nanowebs with 1-3 wt% of ampicillin show high efficiency against gram positive; S. aureus and gram negative; A. lowffii. PVA/ chitosan nanowebs loaded ampicillin have high potential to be applied as wound dressing materials.

**References**


**Acknowledgement**

We acknowledge Department of Materials Technology, and Department of Biotechnology, Ramkhamhaeng University, as well as Department of Chemistry, Faculty of Science and Technology, Rajamangala University of Technology Krungthep University, Bangkok, for technical and instrument support. We also thank AN & A Products Co., Ltd., Bangkok, Thailand for financial support.