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Antimicrobial characterization of silver nanoparticle-coated polyvinyl alcohol/nanochitosan surface by "touch test" method

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The objective of the present study was to fabricate polyvinylalcohol/nanochitosan mat through electrospinning technique followed by impregnation of silver nanoparticles. The silver impregnated mat was evaluated for antimicrobial activity against the pathogens *E. coli, Staphylococcus aureus, Pseudomonas sp,* for antibacterial and *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus* and *Mucor sp,* for antifungal activity respectively. The physico-chemical properties of the mat was characterized using advanced analytical techniques such as Fourier transform infrared spectroscopy (FT-IR) and X-ray diffraction studies (XRD). The preliminary studies supported that the silver nanoparticle impregnated mat exhibits good antimicrobial property with excellent biocompatibility which made them to plausible potential candidates for biomedical applications.

Keywords: Silver nanoparticle, nanochitosan, antimicrobial efficacy, pathogens, crystallinity.

Introduction

With the rising importance of green chemistry the use of biopolymers, chitosan has received extensive attention for its unique properties of biodegradability, biocompatibility, which endow them opportunities for various applications including biomedical, industrial and chemical industries[1].

Another important material play a significant role in biomedicine field is silver nanoparticle because of its excellent antibacterial, antifungal, antiviral, anti-angiogenisis, antiplatelet activities. Silver products are well known for their strong inhibitory and bactericidal effects, thus it has been used for many years to prevent and treat various diseases and infections[2]. Based on previous studies, we chose to use polyvinyl alcohol as a co-spinning agent due to its excellent electrospinning characteristics, its ability to form ultrafine fibers, its linear structure with flexible chains, its biocompatibility and degradability, its solubility in aqueous media, its crosslinking ability and its capability to form hydrogen bonds with other natural macromolecules[3].

Hence in our present work we aimed to fabricate nanofiber mat of polyvinyl alcohol/nanochitosan by electrospinning followed by impregnation with silver nanoparticles prepared by reducing silver nitrate by sodium borohydride. The antimicrobial activity was compared and reported.

Experimental

Chemicals and reagents:

Chitosan with 92% of deacetylation was procured from India Sea Foods, Cochin, Kerela. Polyvinyl alcohol was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai. Sodium borohydride was supplied by Sisco Research Laboratories Pvt. Ltd., Maharashtra and silver nitrate from Scientific Advance Company, Chennai. Deionized water was used for the preparation of PVA/NC polymeric solution. The entire chemicals used in the study were of analytical grade, and were used without any further purification.

Preparation of nanochitosan and fabrication of nanofiber mat using electrospinning setup:

Nanochitosan was prepared by ionotropic gelation method using TPP as crosslinker. 400 mg of chitosan was dissolved in 35 mL of 2% (v/v) acetic acid prepared using deionized water and the solution was stirred well for 45 min. Then to the prepared chitosan solution, 0.5 g of sodium tripolyphosphate dissolved in 10 mL of deionized water was added dropwise with rapid stirring to obtain milky emulsion and the

nanochitosan formed was washed several times in deionized water.

The prepared nanochitosan solution was added to 5% polyvinyl alcohol and stirred continuously for 48 h in magnetic stirrer to get homogeneous polymeric solution. The solution was loaded into 2 ml syringe and spinned in ESPIN NANO. Electrospinning of the solution was performed at the following operating parameters: applied voltage of 20 kV, solution flow rate of 0.55 mL/h, and the tip to collector distance of 12 cm. Fabricated mat of PVA/NC was impregnated with silver nanoparticles (by reducing silver nitrate with sodium borohydride) and examined for antimicrobial studies using touch test method.

Preparation of silver nanoparticles:

30 ml of 0.02 *M* sodium borohydride was stirred in magnetic stirrer for 30 min in ice cold bath. To the above solution 2 ml of 0.001 *M* silver nitrate was added dropwise with constant stirring. Yellow colored solution of silver nanoparticles was obtained. The nanofiber mat was impregnated with the obtained silver nanoparticle solution and used for further evaluation against pathogens to carry out antimicrobial studies.

Results and discussion

FTIR spectroscopy:

Fig. 1 shows the FT-IR spectrum of nanochitosan and PVA/NC mat. The spectrum of nanochitosan exhibits the prominent broad absorption peak at 3385.92 cm^{-1} for the OH, N-H group[4], at around 1324.83 cm^{-1} is assigned to P=O stretching vibration and at about 1038.87 cm^{-1} is due to P-O stretching vibration. These noticeable peaks indicate the conversion of chitosan in nanorange by chemical crosslinking with TPP was successfully achieved.

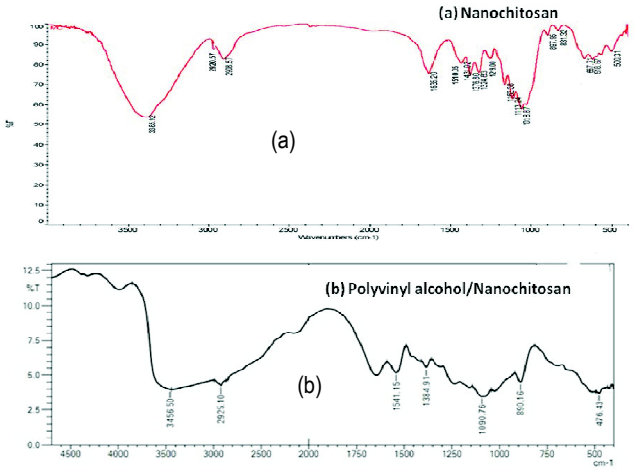

Fig. 1. FT-IR spectrum of (a) nanochitosan and (b) polyvinyl alcohol/nanochitosan mat.

On comparing with nanochitosan, in the spectrum of PVA/NC mat there appears a significant shift in almost all the important absorption bands such as OH, N-H stretching vibrations will observed at 3476.75, P=O and P-O stretching vibration were seen at 1384.91 cm^{-1}, 1081.12 cm^{-1} respectively[5]. The presence of CH stretching attributing to CH_2 and CH_3 group[6] were obtained at 2924.13 cm^{-1} and C-O stretching vibration from the primary alcohol at 1384.91 cm^{-1} corresponds to the OH stretching (O=P-OH) confirms the presence of nanochitosan. Hence the FT-IR spectral details confirm the effective blending of individual homopolymer polyvinyl alcohol and nanochitosan was achieved during electrospinning.

X-Ray diffraction studies (XRD):

The XRD pattern of PVA/NC mat was shown in Fig. 2 and the crystallite size values was calculated using Scherrer equation and it was tabulated in Table 1.

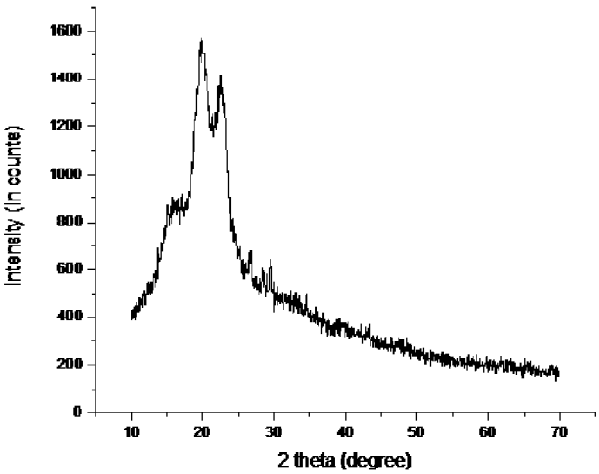

Fig. 2. XRD pattern of polyvinyl alcohol/nanochitosan mat.

Table 1. Crystallite size value using Scherrer equation

2θ	Miller indices	Crystallite size (nm)
15.8	(120)	14.30
17.2	(211)	15.58
20	(220)	18.16
22.6	(311)	20.58
28.4	(322)	26.07
29.6	(331)	27.22

For the PVA/NC mat the graph recorded depicts, intense crystalline band at 2θ = 20, 22.6 and shoulder peaks at 2θ values of 15.8, 17.2, 28.4, 29.6. Karupasamy *et al.* (2012) reported in his work, the diffraction peak of nanochitosan were obtained at 15.19° and 23° corresponding to (120) and (220) planes which was in agreement with the prepared PVA/NC mat[7] and the crystallite size determined using Debye-Scherrer formula was found to be in the range between 14 to 27 nm confirming the reduced crystallite size was attained with simultaneous evaporation of solvent and solidification of elongated nanofibers during electrospinning facilitating amorphous nature to the mat.

Antimicrobial study:

Antimicrobial activity of prepared mat was carried out using touch test method by adapting Mueller Hinton Agar (MHA) medium for bacterial species and Sabouraud Dextrose Agar (SDA) for fungal species and the results were shown in Fig. 3. The zone of inhibition values for antibacterial and antifungal activity was tabulated in Tables 1 and 2 respectively.

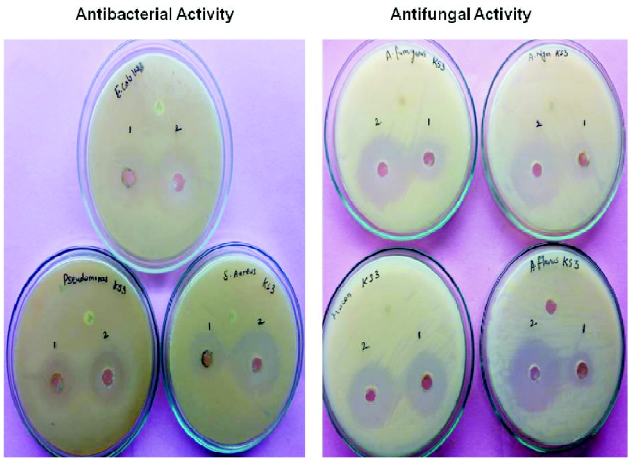

Fig. 3. Antimicrobial activity of PVA/NC (1) and PVA/NC impregnated with Ag nanoparticles (2).

Table 2. Antibacterial activity of PVA/NC (1) and PVA/NC impregnated with silver nanoparticles (2)

	Antibacterial activity Zone of inhibition (mm)		
Sample	*E. coli*	*P. aeruginosa*	*S. aureus*
PVA/NC	16	13	12
PVA/NC with Ag	34	18	31
Control	20	22	20
		(Tetracyline)	

Herein, the result showed that silver impregnated PVA/NC nanofiber mat have higher antimicrobial activity than PVA/NC due to the synergetic effect of Ag^+ nanoparticle and NH_3^+ ions in the nanochitosan. Both Gram-positive and Gram-negative bacteria were effectively inhibited by releasing reactive oxygen species that disrupt robustness of cell membrane, physical interaction of silver nanoparticles and NH_3^+ of nanochitosan into the microbe provoking damage in the cell wall thereby altering permeability leads to cell death[8].

Table 3. Antifungal activity of PVA/NC (1) and PVA/NC impregnated with silver nanoparticles (2)

	Antifungal activity Zone of inhibition (mm)			
Sample	*A. fumigatus*	*A. niger*	*Mucor sp*	*A. flavus*
PVA/NC	18	18	25	20
PVA/NC with Ag	40	32	27	40
Control	15	11	13	11
		(Polymycin B sulphate)		

In case of antifungal activity the cell death was due to the entering of nanoparticles of silver and chitosan on the fungal cell membrane will causes changes in mRNA, and internal osmotic imbalance leads to leakage of cell wall end up in death of fungi[9].

Conclusion

In summary, nanofiber mat of polyvinyl alcohol/nanochitosan was successfully fabricated by electrospinning and the parameters were systematically studied and optimized. Further the mat was impregnated with silver nanoparticle and has been tested for antimicrobial studies using touch test method. For antibacterial study the maximum bactericidal effect was observed for Gram-negative bacteria *E. coli* due to synergetic effect of silver ions and NH_3^+ ions. Almost all the fungal species chosen in the current study shows an effective antifungal activity suggesting this material was effective to be used for food and biomedicine industries.

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