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Research Article

DEVELOPMENT AND CHARACTERISATION OF CHITOSAN/PVA NANO FIBER FOR HEMORRHAGE CONTROL DRESSING

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ABSTRACT

A wound is a type of physical trauma where in the skin is torn, cut or punctured. Wound may be broadly classified in to open wounds and closed wounds. Incisions or incised wounds, irregular tear-like wounds, superficial wounds, Puncture wounds and penetration wounds falls under the open wound category. Bruises, blood tumor, crush injury, Chronic and acute or traumatic wounds are some of the few examples for closed wounds. In this research work chitosan and PVA with different concentration were used to produce nano fibre film using electro spinning process. The effectiveness of the nano fibre coated with chitosan was analyzed by using SEM, FTIR, antibacterial test, in vitro drug release test and tissue responses in chick nano-film membrane (CNM) test. The result confirms that the chitosan/PVA nano fibre electro spun film can be effectively used for hemorrhage control dressing.

Keywords: trauma, wound, nano fibre, membrane, chitosan, antibacterial test

INTRODUCTION

A wound is a type of physical trauma where in the skin is torn, cut or punctured. Wound may be broadly classified in to open wounds and closed wounds. Incisions or incised wounds, irregular tear-like wounds, superficial wounds, Puncture wounds and Penetration wounds falls under the open wound category. Bruises, blood tumor, crush injury, Chronic and acute or traumatic wounds are some of the few examples for closed wounds. Wound healing, or repair, is an intricate process in which the skin (or another organ tissue) repairs itself after injury. Wound healing take place by four phases namely inflammatory phase, proliferative phase, contraction phase and maturation and remodeling phase. In this research work a wound healing material has been developed by using poly vinyl alcohol (PVA), chitosan and acetic acid. The PVA is mixed with chitosan in the presence of acetic acid to form the base solution. This blended solution is then converted in to nano film material using electro spinning technique. The solution is prepared with various combination of PVA and Chitosan for producing nano film.

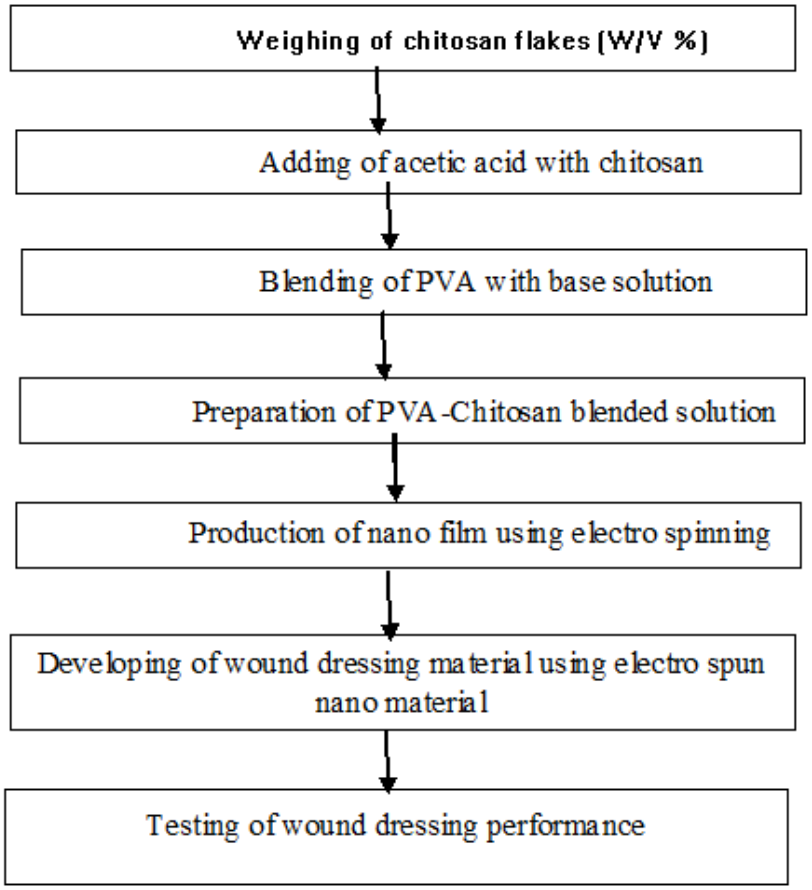
Literature Review

The bio polymer for medical application largely depends on the future needs of our civilization. The use of new fibers for healthcare textile application on recent advances in tissue engineering, drug delivery- alginate, chitin/chitosan and their derivatives present a novel and useful class of biomaterials [1]. Electrospinning method as a simple technique for fabricating tissue engineering scaffolds. Electro spinning webs have a biocompatibility and high surface area. Therefore, the electro spinning technique can be used in the medical applications [2] Chitosan and sodium alginate feature good biocompatible and non-toxic and are both widely applied as bio-medical materials in bio-technology. In addition, chitosan is also antibacterial and is able to accelerate healing [3]. The poly ethylene oxide blended with alginate and electro spun made as scaffolds to support human dermal fibroblast cell attachment [4]. polyvinyl alcohol, continuous fibers were successfully electro spun from PVA solutions [5]. Nanofiber scaffolds shown to be promoters for tissue cell adhesion and encapsulates for drugs [6]. Wound care products were developed using poly vinyl alcohol blended with chitosan. PVA/Chitosan wound dressings have high moisture vapor transmission property, good antimicrobial activity and do not have any cytotoxicity effects [7]. Nano fibrous membranes are highly soft materials with high surface-to-volume ratios, and therefore can serve as excellent carriers for therapeutic agents or accelerate wound healing [8]. The high moisture absorption and anti-bacterial effects of fibres from zinc or copper alginate will allow the production of a new generation of dressing materials [9]. These bandages could be made such that they contain bioactive ingredients, such as antimicrobial, antibacterial, and anti-inflammatory agents, which could be released to the wounds enhancing their healing [10]. Chemical and physical modifications of chitosan influence its biocompatibility and biodegradability [11].

MATERIALS AND METHODS

For developing of wound dressing materials Chitosan poly vinyl alcohol and acetic acid were used. Solutions were well prepared by using magnetic stirrer. Electro spinning machine is used to produce the nano film of thickness 0.05mm and it is deposited on the aluminium foil covered with spun bonded polypropylene nonwoven material of 15GSM.

The method used to produce the nano film wound dressing material is as shown in the flow chart below



The biopolymer of various Strength has been prepared after dissolving in acetic acid and distilled water. The solution has been stirred thoroughly to form a homogeneous solution and stored for 12 hours for profanation process. The PVA solution blends further stirred for 2 hours using the magnetic and electronic stirrer. The sample prepared for doing the trial study have been shown in table 1.

Table 1. Solution preparation in different ratio

Sample no.	Chitosan			PVA			Acetic acid 20%
	Total volume of solution (ml)	Weight (gms)	Strength (%)	Total volume of solution (ml)	Weight (gms)	Strength (%)	
CH/ PVA 70/30	10	0.2	2%	10	0.7	7%	2ml
Chitosan/ PVA 50/50	10	0.2	2%	10	0.7	7%	2ml
Chitosan/ PVA 30/70	10	0.2	2%	10	0.7	7%	2ml

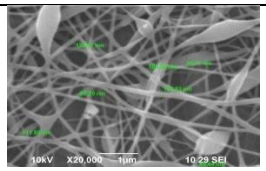
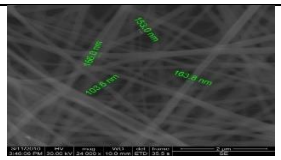
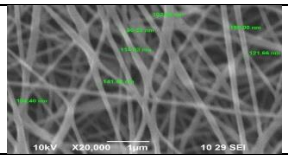
The applied voltage for the process is 19 kV, 20 kV, and 22 kV respectively. The collection distance between the needle tip to the collector (aluminum foil sheet) is fixed as 15 and 16 cm. The flow rate from the needle orifice is fixed as0.02 and 0.05ml/hour. The Chitosan, sodium alginate and calcium alginate solutions with concentration of 1w/v% and 2w/v% respectively were used for electro spinning. The PVA solutions with concentration of 7w/v% respectively were used for electro spinning. The concentration of acetic acid is 1% v/v and 2% v/v respectively were used for electro spinning. The morphology of the electro spun fibers of chitosan, calcium alginate and sodium alginate can be observed under a scanning electron microscope after platinum coating by an ion sputtering with 15 mA for 4 minutes for each sample. The antibacterial effect of the Nano film were determined qualitatively by agar diffusion plate test using EN ISO 20645:2004 method against Staphylococcus aureus ATCC 6538 and Escherichia coli ATCC 10229. The antimicrobial activity was quantitatively evaluated using AATCC 100-2004 test method against the standard bacterial strain which gets effectively inhibited by EN ISO 20654:2004 method. The drug release of Biopolymers and PVA grafted nano film (0.2 mg) were tested by placing in separate screw cap tubes containing 3ml of artificial blood (pH-6.4±0.1) and incubated at 37˚C for specified hours. After incubation for specified time intervals, the supernatant was

removed, and the medium in the test tube was replenished with fresh PBS (phosphate buffer saline). The amount of drugs released from both the specimens was measured at 276nm using UV-spectrophotometer. Similar procedure was followed for all the nano film using different concentration of the polymers.

RESULTS AND DISCUSSION

Surface Morphology Analysis

Table 2 Surface Morphology of Chitosan and PVA Electro spun fibre

Sample code and specification	SEM Image	Mean fibre Diameter nm
CH/PVA-50/50% Chitosan= 2.0% Pva = 7.0% Feed rate = 1.0ml/hour		110
CH/PVA-30/70% Chitosan= 2.0% Pva = 7.0% Feed rate = 0.5ml/hour		146.2
CH/PVA-20/80% Chitosan= 2.0% Pva = 7.0% Feed rate = 0.5ml/hour		118.2

From the SEM analysis (table 2) we can conclude the fibre diameter spun from electro spinning depends on the feed rate and the ratio of blending of chitosan and with PVA. As the ratio of PVA increases compared to the chitosan the fibre diameter reduces drastically. As the polymer solution concentration increased, the beads and breaking fibres appeared, because of the cohesive nature of the high viscosity solution. When the polymer solution concentration was increased the average fibre diameter also has been increased proportionately from 150 nm to 400 nm. At low viscosity, droplets and breaking fibres are formed, which coalesced so as to constitute an electrospray, but as the solution viscosity increased fibres began to form, also, the formation of beads was suppressed as the solution viscosity increased. The fibre formation ability and its morphology were closely related to viscosity of the solution.

FTIR Analysis Chitosan/PVA Nano film

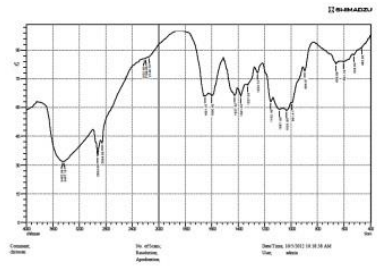


Figure 1 FTIR spectrum of raw state chitosan

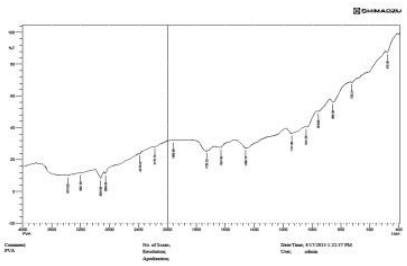


Figure 2 FTIR spectrum of raw state PVA

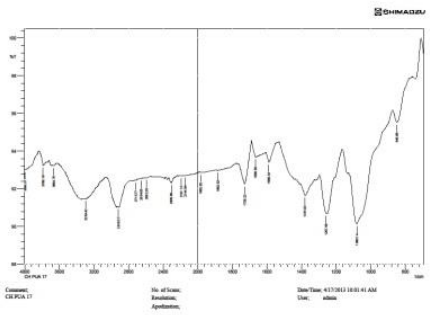


Figure 3 FTIR spectrum of Chitosan/ PVA nano film

The PVA blended chitosan nano film (Figure 2 to 4) shows the distinct C-H stretching exhibited in the at 3452-3294 and 2924-2916 cm^{-1} due to vibration of alkanes rather than raw state chitosan. The peak 2198-2191 cm^{-1} shows the distinct slightly C-C stretching due to vibration of alkyne. The peak 1253-1257 cm^{-1} shows C=C stretching due to alkene and aromatic ring. The peak 1381 cm^{-1} there is no significant difference while comparing raw state chitosan to chitosan/PVA nano film in the C-O band. The peak 1728 cm^{-1} there is no significant difference while comparing raw state PVA to chitosan/PVA blended nano film in the C=O band (amides, ketones, carbolic acid, esters).

Antibacterial test by agar diffusion method

The antibacterial effect of the different concentred chitosan nanofiber film were determined qualitatively by agar diffusion plate test using EN ISO 20645:2004 method against Staphylococcus aureus ATCC 6538 and Escherichia coli ATCC 10229. When effective antibacterial activity was determined against the used bacterial pathogens, AATCC 100-2004 test method was used to analyse reduction in bacterial counts for quantitative determination.

Table 3 Inhibition zone by Agar diffusion method

PVA-chitosan nanofiber matrix	Inhibition zone (mm)	
	***S. aureus* ATCC 6538**	***E. coli* ATCC 10229**
CH/PVA 50/50%	0 mm and no growth reduction	0 mm and no growth reduction
CH/PVA 30/70%	0.7±0.2 mm[a]	0 mm and slight growth[b]
CH/PVA 20/80%	1.1±0.1 mm[a]	0.4±0.2 mm[b]

Table 4 Bacterial Reduction Percentage by ATCC 100 method

PVA-chitosan nanofiber matrix	Reduction of bacteria (%)	
	***S. aureus* ATCC 6538**	***E. coli* ATCC 10229**
CH/PVA 50/50%	87	69
CH/PVA 30/70%	93	74
CH/PVA 20/80%	95	81

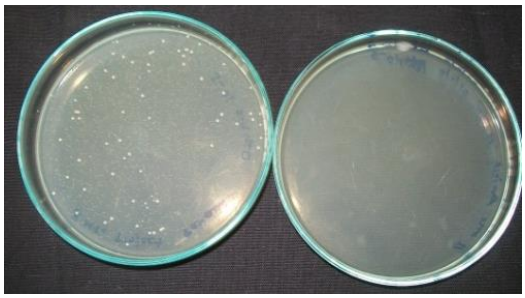


Figure 4 :10^{-4} dilution plates of E. coli and S. aureus of the specimen

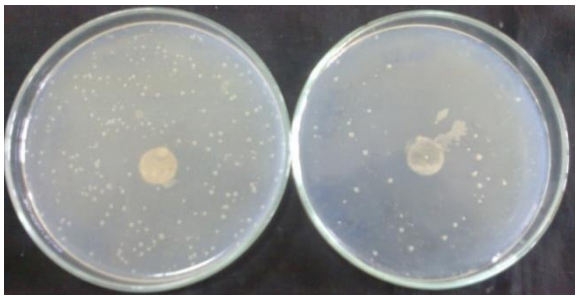


Figure 5: 10^{-5} dilution plates of E. coli and S. aureus of the specimen

The antibacterial activity analyzed by EN ISO 20645:2004 test method of the coated nano film was expressed as zone of inhibition diameter as an average (± standard deviation) of duplicate determination. The results tabulated in Table 3 and 4 indicate the bio efficacy of the used polymer. The test exhibits good antibacterial activity against *S. aureus* ATCC 6538 and limited effect on *E. coli* ATCC 10229 (figure 4 and 5). Even though coated nano film was effective against *S. aureus* ATCC 6538 than *E.* coli ATCC 10229, the bacterial reduction percentage was analyzed for both the pathogens using ATCC 100-2004. The direct seeding of microbial pathogens on the nano film enhances the microbial colonization on the film surfaces. The reduction percentage of *S. aureus* ATCC 6538 and E. coli ATCC 10229 in electro spun samples were found to be in increasing fashion as the concentration of chitosan used for electrospinning increases.

Table 5 In Vitro Release of Chitosan

Time interval of drug release analysis (h)*	Chitosan release profile (µg)*	Release profile of chitosan from nanofilm (µg)*		
		Chitosan/PVA-20/80%	Chitosan/PVA-30/70%	Chitosan/PVA-50/50%
24	20±0.4	I20±0.5	I29±0.3	135±0.4
26	50±0.3	140±0.7	148±0.7	152±0.2
28	70±0.1	145±0.4	154±0.2	165±0.6
48	100±0.6	175±0.8	180±0.1	185±0.5

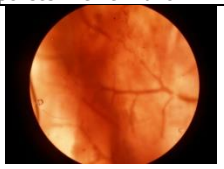
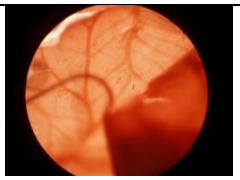
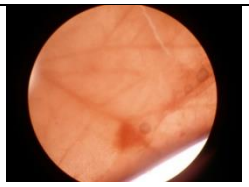
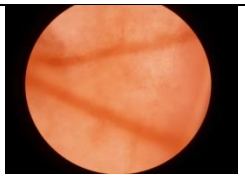
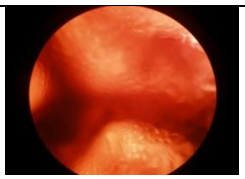
The release profile of chitosan revealed that when chitosan concentration increases, there will be an increase in release profile (table 5). This is due to the increase hydrophilicity of the spun nanofibers with more covering area. When compared the chitosan particles at this pH had very low release profile. The chitosan in its original polymeric form is highly insoluble at the used pH. But the mild acid dissolved chitosan polymerized to a semisolid structure can show release profile at the used mild acidic pH. It is observed that the Chitosan percent increase, the rate of drug release also increased.

Tissue responses in chick nano-film membrane (cnm) test for bio compatibility

Nine day embryonated eggs were handled to determine position of air sac. Nano-film membranes were punctured and a window

was cut in the shell and membrane. A small piece specimen (1.5mm x 1.5mm) was carefully placed on the Nano-film membrane of eggs. Windows were then sealed with heated adhesive tape, incubated for 7 days at 37˚C. Nano-film membranes were carefully dissected out and immediately placed in 10% formal – saline solution. After 24 hours fixation, implants were teased away from underlying Nano-film membranes which were then trimmed, paraffin embedded and prepared for staining by hematoxylin and eosin.

Table 6 Tissue Responses In Chick Nano-Film Membrane (CNM)

Sample particulars	Tissue responses in chick nano-film membrane
Chitosan/PVA Nano film -20/80%	
Chitosan/PVA Nano film 30/70	
Chitosan/PVA Nano film 50/50	
Negative Control (0.1 % NaCl)	
Positive Control (0.1 % NaCl)	

The specimens of Electros pun Nano film samples with 0.1 % NaCl as negative control and 0.1% NaOH as positive control were implanted (table 6) in the nine-day old embryonated chick eggs by placing it on the nano film membrane portion and incubated for 7days. During and after the exposure period (9, 12 and 17 days), the eggs were observed for hypersensitive reactions-edema and erythema. No such reactions on the surface of the chicks were observed after the specified incubation period in all the specimens except positive control which showed degeneration of blood vessels. The stained membrane showed the normal blood vessel formation without any pleuritic symptom and hence the samples prepared in this experimentation can be considered biocompatible.

CONCLUSION

The electro spinning process parameters applied voltage, flow rate, distance between the needle tip to the collector also have a greater influence in the fiber diameter. From these investigations we conclude that as the chitosan strength increase the fiber diameter also increases and vice-versa. The increase in voltage leads to an increase in charge density, thereby causing the accelerate faster while encouraging more stretching and produce thinner fiber. The antibacterial test shows that the chitosan and PVA blended nano film has the good antibacterial activities. Both in gram positive and gram negative cultures. The drug delivery of PVA blended chitosan nano film shows excellent result in PBS. The biocompatible test shows the specimens (Electro spun Nano film samples with 0.1 % NaCl as negative control and 0.1% NaOH as positive control were implanted in the nine-day old embryonated chick eggs by placing it on the Nano film membrane portion and incubated for 7days. During and after the exposure period (9, 12 and 17 days), the eggs were observed for hypersensitive reactions-edema and erythema. No such reactions on the surface of the chicks were observed after the specified incubation period in all the specimens except positive control which showed degeneration of blood vessels. The stained membrane showed the normal blood vessel formation without any pleuritic symptom and hence the samples prepared in this experimentation can be considered biocompatible.

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