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A novel study on wound healing properties of electrospun chitosan-polyvinylalcohol nanofibers.

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ABSTRACT

Special wound care products could be developed using PVA blended chitosan Chitosan, a natural non-toxic polysaccharide obtained from crustaceans, which is produced by deacetylation of chitin. Chitosan shows great antimicrobial activity. Polyvinyl alcohol (PVA) has good film forming capacity. Chitosan/PVA are biodegradable, biocompatible and sustainable polymer. Nanofiber scaffolds were prepared using electrospinning technique which is more convenient form used in wound dressings and other applications. In this current study, the antibacterial nanofiber film will be prepared by electro spinning the solution with the composites of Chitosan /Polyvinyl alcohol. The morphological structure were studied using Scanning electron microscope. The prepared Chitosan/PVA nanofibers diameters were found in the range of 300-500nm in different magnifications with the voltage 20.00 kV. The FT-IR spectra of Chitosan/PVA revealed the formation of intermolecular hydrogen bonds. The weight fractions of fibers were calculated using thermal grsavimetric analysis.The antimicrobial activity was done using disc diffusion method, which showed positive results that assist in process of wound healing.

Keywords: Chitosan, nanofiber, PVA, wound healing.

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INTRODUCTION

In medical applications, nanofibers used as tissue engineering scaffold in drug delivery and wound dressing [1]. The objectives of wound healing includes protection, removal of exudates and improved skin appearance. A wound dressing material must be biocompatible, biodegradable, increase the healing process and have high porosity for respiration [2, 3]. Nanofibers were developed using template synthesis, drawing, phase separation, electrospinning, self-assembly and so forth [4]. Of the following, electrospinning is a versatile method to fabricate micro and nanofibers [1, 5].

The electrospun nanofibers have high surface area to volume ratio, oxygen permeable porosity, different pore sizes and wound dressing material which initiates cell adhesion, migration and fibroblast proliferation [6]. In this process, when sufficiently high voltage is applied to a liquid droplet, the liquid gets charged due to electrostatic forces which draw charged threads of polymer solution and is ejected from the tip. Charged jets move towards the collector, the fiber mat is collected once the solvent gets evaporated [7]. By adjusting the electrospinning parameters the morphology of electrospun nanofibers can be easily controlled [8,9]. The factors influence the formation of nanofibers by various parameter, which includes

i. Solution parameters consist of conductivity, surface tension and solution viscosity.
ii. Process parameters consist of voltage, distance between tip and collector, flow rate and induced electric field by the collector.
iii. Systemic parameters, includes Polymer type, Molecular weight and solvent used [10, 11].

Chitosan is a poly-cation and copolymer of N- acetyl -D-glucosamine (GlcN) and D-glucosamine produced by deacetylated form of chitin [12, 13]. Chitosan is a polysaccharide, has antibacterial activity which is used for wound dressing. These are biocompatible, biodegradable and bioactive in nature [14, 15]. Chitosan promotes hemostasis which helps in tissue regeneration [16]. Due to its excellent properties of binding toxic heavy metal ions, it inhibits the growth of microorganisms [17]. Due to active amino and hydroxyl groups, chitosan has polycationic chelating and film forming capacity [18]. The antimicrobial properties of chitosan results in polycationic nature of chitosan arising from protonation of $-NH_2$ group on the chitosan backbone. Interaction of polycationic chitosan with anionic groups on the cell surface resulting in the increase of membrane permeability and facilitates leakage of chitosan or essential nutrients in the cell [19].

To prepare chitosan-based materials, chitosan is to be dissolved in weak acids. The structure, size of acids as counter ions, influences interactions between intra and intermolecular of chitosan, due to the pH and type of acid and material properties have shown some changes [20].

Polyvinyl alcohol (PVA) is biocompatible, biodegradable and hydrophilic synthetic polymer used in various biomedical applications such as bone implants and artificial organs [21]. It is used as temporary skin covers and as drug-delivery systems, wound dressings, dialysis, skin and intervertebral discs, artificial cartilage and cardiovascular devices [22, 23, 24]. The development of PVA hydrogel exhibits exemplary transparency and anti-electrostatic process, when it undergoes crosslinking [25]. The nanofiber blends of PVA and Chitosan have been fabricated by electrospinning, since PVA has good fiber forming capacity [26]. The repelling interactions of chitosan chain are reduced and the molecular interactions are improved, when PVA is added to chitosan solution [27]. The blends of Chitosan/PVA seems to be viable to obtain nanofibers via electrospinning. The nanofibers may be cross-linked using physical methods such as dehydrothermal treatment, UV irradiation and chemical cross-linkers such as formaldehyde, glutaraldehyde, carbodiimide and dextran dialdehyde [27,28].

The main objective of this study is to synthesize chitosan/Polyvinyl alcohol blends of electrospun nanofibers. The prepared nanofibers are characterized for the morphological structure (SEM analysis), the functional groups present (FTIR analysis), the thermal degradation by Thermogravimetry analysis which further aids in the healing process of wound. The antimicrobial activity was done using agar disc diffusion method.

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MATERIALS AND METHODS

Chemicals

Chitosan with molecular weight (MW1526.454- deacetylated chitin) ,PolyVinylAlcohol (99% hydrolyzed) were purchased from Sigma Aldrich CO., Glacial Acetic acid and Avantor Performance Materials India Limited – RANKEM Laboratory reagent. The bacterial strains were obtained from Lifeteck Research Institute, Chennai. Mueller Hinton Agar (MHA) was purchased from Hi-Media Laboratories, Chennai. All other chemicals used were of analytical grade.

INSTRUMENTATION

ELECTROSPINNING EQUIPMENT AND OPTIMIZATION PROCESS

The chitosan/PVA solution was loaded onto the plastic syringe tube which was fitted on to a needle with 21G tip. The blends were then subjected to the electrospinning equipment usingb high voltage power supply.

SCANNING ELECTRON MICROSCOPE (SEM) ANALYSIS

Morphological characterizations of the electrospun nanofibers made by Chitosan Polyvinyl alcohol were studied using Quanta 200 FEG scanning electron microscope (SEM). The nanofibers prepared was cut into small piece of 10mm diameter and placed onto the clean aluminum foil sheet which was stabbed to the stub. The setup is air dried and then vacuum is created by suction pump and then subjected for SEM analysis.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

The electrospun nanofibers synthesized were dried for an entire day and then it can be used for further analysis. A Fourier Transform Infra-Red Spectrometer is used to obtain the infra-red spectra of absorption and emission of the formed nanofibers which indicates the functional groups present in the sample. The advantage of using an FTIR is to simultaneously collect spectral data in a wide spectral range. 10 mg of the electrospun nanofiber sample was collected and subjected to FTIR analysis using JOEL spectrophotometer in the diffuse reflectance mode with KBr pellets.

THERMOGRAVIMETRY ANALYSIS

The thermal gravimetric analysis was carried on a Thermo Gravimetric Analyzer. The TGA was done in the range of 30-1000º C under nitrogen atmosphere with a flow rate of 20 ml/ min. 10 mg of sample was taken and dried at room temperature. The graph was plotted with the weight (%) vs. temperatures (˚C).

DISC DIFFUSION METHOD

By disc diffusion method on Muller Hinton agar (MHA) medium the antimicrobial activity of the nanofibers was determined. The MHA medium was poured on to the petri plate. When the medium gets solidified the inoculums were spread eventually on plates using the sterile swab. The discs were placed in MHA plates along with a control antibiotic. The plates were incubated at 37ºC for 24 h. Finally,the diameter of zone of inhibition is measured[29].

METHODOLOGY

Preparation of Chitosan Stock Solution (2-6 %)

4 gm of Chitosan powder is dissolved in 100 ml of acetic acid.

Preparation of Polyvinyl Alcohol (PVA) Stock Solution (14%)

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14 gm of PolyvinylAlcohol is dissolved in 100 ml of deionized water and kept in magnetic stirrer for 1 hour at 1300 rpm to prepare 100 ml of 14% of PVA solution. Different proportions of chitosan and PVA solution like 2:8, 3:7, 4:6, 5:5 and 8:2 were prepared by blending the solutions. And kept in magnetic stirrer for 2 h and taken for electrospinning process.

RESULTS AND DISCUSSION

ELECTROSPINNING PROCESS

Chitosan/ Polyvinyl Alcohol(PVA) blends were prepared in different proportions with varied ratios like 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2. Because the polymer PVA has good film forming capacity, which is the main composite for synthesis of nanofibers. In the ratio 2:8 the chitosan content is too low and the PVA content was viscous. Due to the high viscosity of the solution, the blends got coagulated and jet formation was poor in the spinning process, where as in other ratios such as 4:6, 5:5,6:4, 7:3, 8:2, the polymer content was less and chitosan was high. when the fibers were produced the solution in the syringe started spraying and no uniform fibers were seen. Out of these, the fibers were fine film with chitosan-PVA ratio 3:7.

The chitosan/PVA nanofibers were successfully synthesized using electrospinning process. The flow rate was varied from 0.5 ml/h, 1.0ml/h, 1.5ml/h, 2.0ml/h and 2.5 ml/h and voltage from 15 kV to 25 kV. Different trials were carried out and optimized to get the final value of 1.0 ml/h, which produced continuous nanofibers at 22 kV.

SCANNING ELECTRON MICROSCOPE (SEM) ANALYSIS

The current studies reveal that the synthesized electrospun nanofiber undergone SEM analysis found to represent nanofibrous surface. The SEM image of Chitosan-PVA nanofibers were shown in the following figures which represent the nanofiber in the range of 500nm Fig 1(a), and 300nm Fig 1(b) with voltage of 20.00 kV. The nanofibers surface helps in the increased surface area action that would help in effective wound healing process [30].

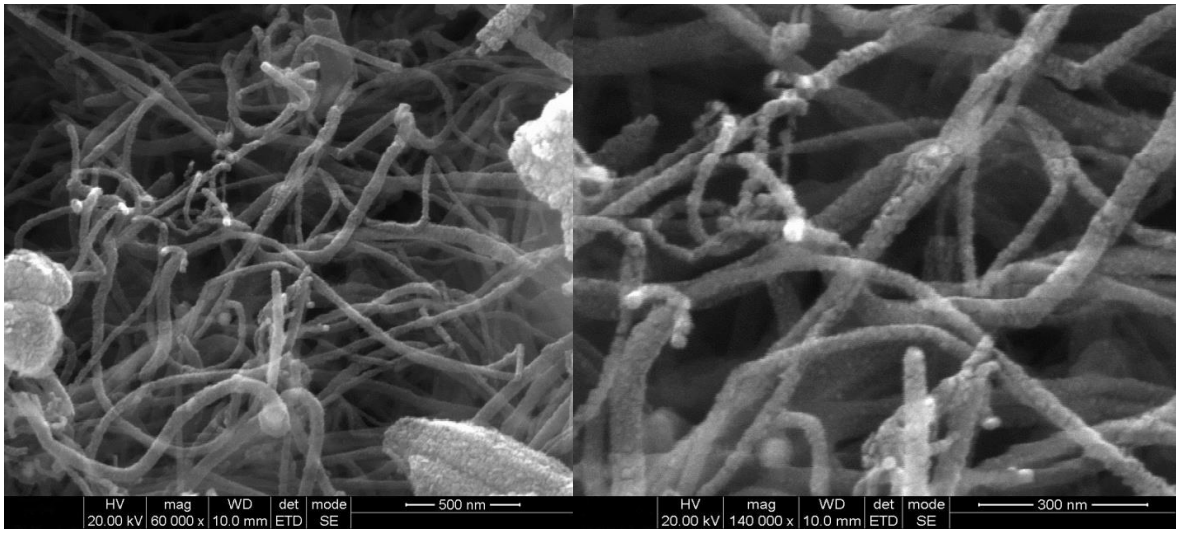

Fig 1(a) **SEM image of chitosan-PVA (3:7) electrospun nanofiber @500nm**
Fig 1(b) **SEM image of chitosan-PVA (3:7) electrospun nanofiber @300nm**

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

Figure 2 indicates the presence of different functional groups present in the Chitosan-PVA nanofibers using Fourier Transform Infrared (FTIR) Spectroscopy. FTIR results for Chitosan-PVA nanofiber at the ratio of 3:7 indicates the medium bending vibrations at 3390.43cm^{-1} might be due to the presence of –NH_2 groups, which is mainly found in chitosan. The strong-CH stretching vibrations are shown at 2918.93cm^{-1}, which is

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present in PVA. The strong bending vibrations at 1244.01 cm^{-1} and 1090.20 cm^{-1} are due to –CO functional groups found in chitosan. The absorption peaks at 1731.75cm^{-1} and 1401.20cm^{-1}, which indicates the presence of C=C and C=N groups respectively.

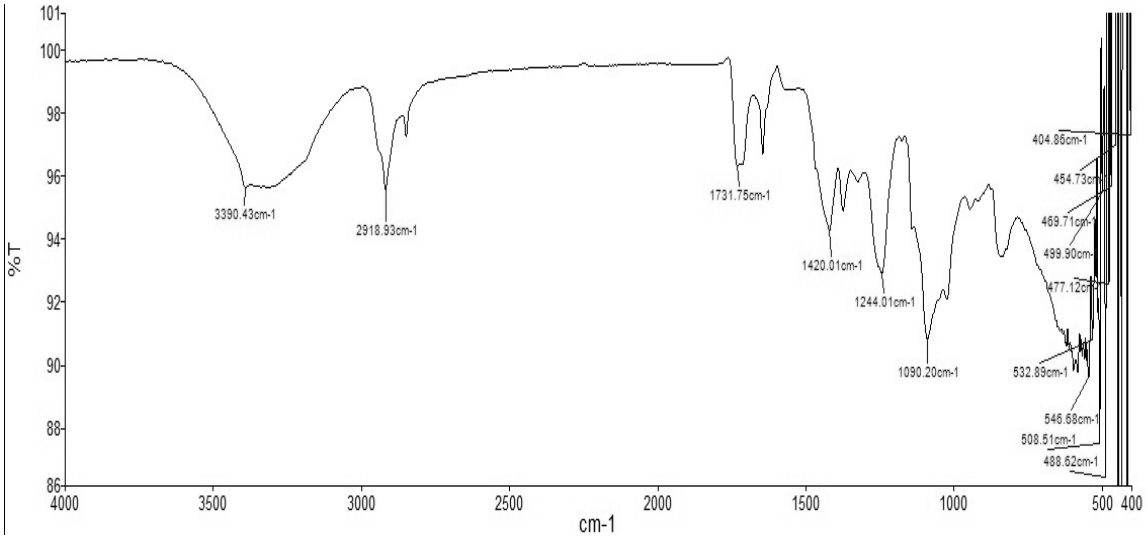


Fig 2 (b) FTIR spectra of the chitosan-PVA (3:7)

THERMOGRAVIMETRY ANALYSIS

TGA analysis of Chitosan-Polyvinyl Alcohol nanofibres was shown in Fig 3. The first step of all polymers is an initial weight loss due to the moisture content. Two weight loss drifts were observed in chitosan-PVA nanofibers. On the chitosan-PVA polymers, the first step of decomposition of nanofibers was at around 123.4˚C. After the loss of water the second decomposition step was at around 352.4˚C. When the test ended at 747.3˚C the sample lost its moisture content, showing the residual mass of about 46.23%.The reported literature by Santos [31] shown the residual mass of 29.7±0.5%. This showed that chitosan-PVA nanofibers possess high thermal stability to withstand high temperatures.

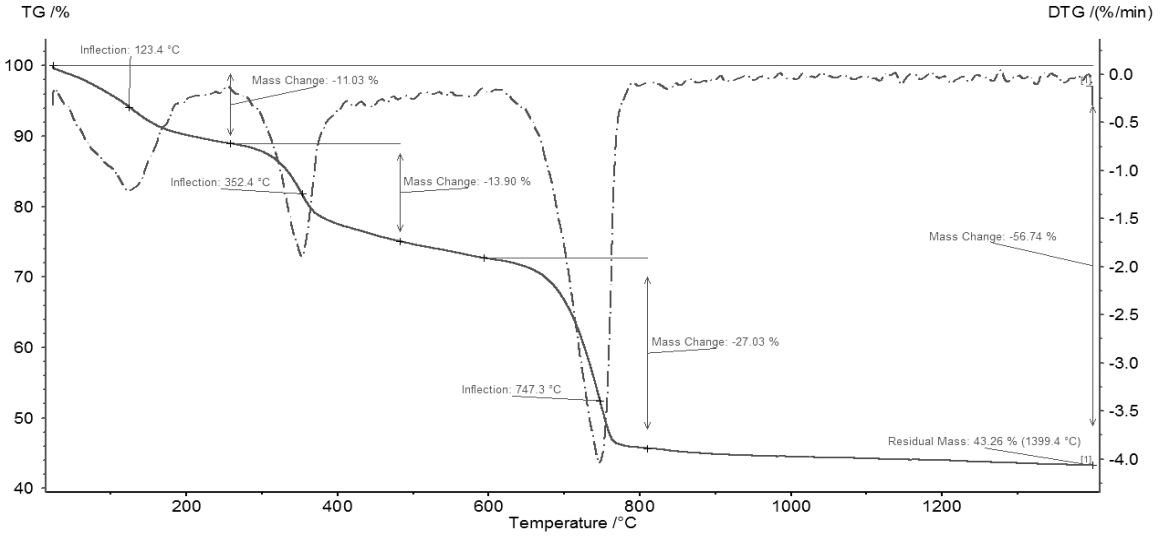
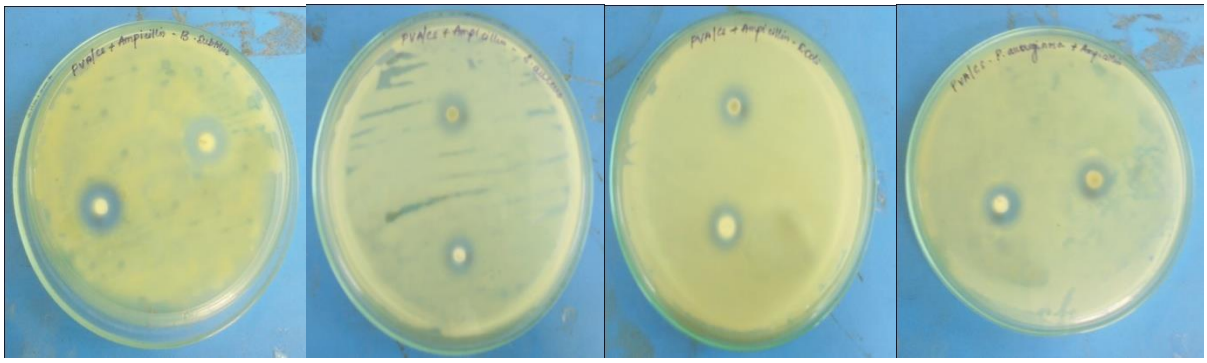


Fig 3 Thermo gravimetry Analysis (TGA) of Chitosan-PVA nanofibres (3:7) at heating rate of 10˚C/min.

DISC DIFFUSION METHOD

Antibacterial activity of Chitosan-PVA nanofibers was checked against *gram positive and gram negative bacteria* using disc diffusion method. The inhibition range in microorganisms were positive using

Chitosan-PVA nanofibers. When compared with antibiotic, the zone of inhibition for chitosan-PVA shows better activity than antibiotic as shown in Table 1. The reported literature done by Unnithan was used to compare the antibacterial activity of Polyurethane(PU) dextran- drug nanofiber with PU dextran and prestine PU discs. It was concluded that Chitosan-PVA shows better antibiotic activity, which helps in effective wound healing.



B. subtilis ***S. aureus*** ***E.coli*** ***P. aeroginosa***

Fig 4 Antibacterial activitiy of Chitosan-PVA nanofibers

Table 1. Antibacterial activity of Chitosan-PVA nanofibers against *Staphylococcus aureus, Bacillus subtili,s, E.coli, Pseudomonas aeroginosa.*

Organisms	Zone of Inhibition (mm)	
	Sample	Antibiotic (1mg/ml)
Bacillus subtilis	13.8	16
Staphylococcus aureus	16	13
E.coli	17.5	13
Pseudomonas aeroginosa	14	15

CONCLUSION

Since chitosan is biocompatible and non-toxic polysachharide, was taken for the present study. Chitosan nanofibers were successfully synthesized along with PVA polymer by the process of electrospinning. The chitosan-PVA nanofibers were synthesized and the structural morphology clearly indicates the presence of nanofibers, which is shown evitable through Scanning Electron Microscopy (SEM) analysis. It was also proved from the FTIR characterization of chitosan-PVA nanofibers, the different peaks were clearly observed for the main functional groups present in chitosan-PVA. The higher thermal stability of chitosan-PVA nanofibers was confirmed through Thermo Gravimetry Analysis. The antibacterial activity of Chitosan-PVA nanofibers was done using agar disc diffusion method and it was confirmed that it was efficient towards microorganisms which helps in better wound healing.

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