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BIO MINERALS FOR HEMORRHAGE CONTROL IN WOUND DRESSINGS

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Abstract: *It has been reported that haemorrhage in trauma accounts for 30–40% of all fatalities as a cause of death.Wound dressings of biominerals are biocompatible, biodegradable, haemostatic and antibacterial biomaterials. Montmorillonite mineral has a hydro absorption which leads to a swelling phase and it stretches open like a highly porous sponge and helps in haemostasis. Along with the minerals, Nano particles are deposited over a nano film and studies are taken. Hence in way of producing nano particles through textile processed wound dressing can possibility of good efficacy so electro spinning technology is used to produce nano film for efficient drug release. The proposed system of wound dressing production in electrospinning is by nano film through various combinations and conditions. The bio - mineral particles that along with the unique properties of nanofibres such as high surface area to volume ratio, porous film, Nano scale fibre diameter, the mineral particles would be useful in absorption of plasma in haemostatic wound care to enhance the rate of blood clotting. Due to their nano particle size in electro spun material had advantage on blood clotting is due to superior cationic exchange property while reacting with blood cells results to faster blood clot with reduced pain in healing. Normal blood clot time takes 240 sec. but in case of nano film it reacts faster and clots the blood 115 sec. This study clearly showed significant alterations in the volume of blood loss as well as the bleeding or clotting time following mineral deposited nano film wound dressing.*

1. Introduction

Wound dressings and devices form an important segment of the medical and pharmaceutical wound care market worldwide. In the past, traditional dressings such as natural or synthetic bandages, cotton wool, lint and gauzes all with varying degrees of absorbency were used for the management of wounds. Mortality from hemorrhagic shock caused by massive bleeding in a wound is preventable. With proper dressing (bandage and compression) of the injured site for bleeding arrest, timely fluid resuscitation for hemodynamics restoration, and definitive hospital management. Electro spinning is a renowned technique for generation polymeric nano fibres that has a very large surface area to volume ratio. Nano fibres obtained from Electrospinning have diverse application scaffolds, bio materials and drug delivery systems. Nanofibres provide large surface area, which makes them suitable for medical textile products such as surgical facemasks or wound dressings, and drug delivery systems. A biopolymer of interest for such applications is chitosan. However, chitosan is not easily electrospun, perhaps due to its polyelectrolyte nature and intrinsically high viscosity. Hence chitosan alone is quite expensive and the film properties are quite delicate. To overcome such drawbacks, previous researchers formed films with sufficient physical strength from polymer and chitosan solutions using polyvinyl alcohol. PVA-Chitosan blend films with 70/30 ratios were prepared by dissolving the polymers. Chitosan nanofibres can be obtained by dissolving chitosan in acetic acid. In this study, The chitosan derivatives namely PVA / chitosan have been developed and then electrospun using different spinning parameters to produce fibre for their application in wound dressings.

2. Materials and Methods

The chitosan was prepared by first dissolving chitosan in acetic acid to yield 1% aqueous acetic acid solution. Then the solution was stirred for 120mins at 60 degree Celsius temperature. Then poly vinyl alcohol solution is prepared in distilled water at 10 % concentration. It is prepared by stirring polyvinyl alcohol with distilled water at 60 degree celsius temperature. Both the solution are prepared separately and combining that in proper proportion for electro spinning solution.

2.1 Preparation of Electrospinning Solutions

The solvent used to form electrospinning solution was polyvinyl alcohol. The required quantity of polyvinyl alcohol and chitosan solution were taken into a bottle and desired amount based on required concentration. Hence 70 % of polyvinyl alcohol and 30% chitosan solution were taken. Then the solution was stirred. The bottle was closed using an airtight lid and then placed in the bath. The solution was left overnight and then used for electrospinning. During left the solution it was settle down and form some viscous ability to electro spinning with low wastage in solution.

2.2 Electrospinning

The electrospinning equipment used consists of an extrusion system (syringe pump), fibre collection system, and high voltage power supply. The extrusion system was used to provide controlled feed rate of the spinning solution. The polymer solution was given a positive field with the help of a high voltage power supply. The terminal wire (positive electrode) from the high voltage power supply was fixed to the extrusion needle (1 mm inner diameter). The system of collection of fibres includes a drum. That drum is covered with copper sheet. The drum is fixed to an electrically isolated wooden frame and connected to a motor and speed controller. A brass ring is attached to one side of the drum. The ring is in contact with a brass bush, which, in turn, is connected to the negative electrode of high voltage power supply. A brass rod is placed on a groove in the drum throughout its length and fixed at one end by the brass ring. The brass rod carries the electric field given by the brass ring. An viscose nonwoven (or any conductive material) is wound over the drum and passes under the brass rod. This is done to maintain the same electric field over the area covered by the conductive foil. The negative end for the system is derived from the high voltage power supply to the brass rod with the help of a connection wire and the brass bush, which is attached to the brass ring. A strip of 10 cm wide viscose nonwoven was mounted securely around the fibre collecting drum. Gauze of the same width was mounted over the viscose nonwoven. Part of the viscose nonwoven was not covered by gauze. By rotating the drum with low speed (1-3 m/min), a nanofibres layer was spun over the gauze and the viscose nonwoven without any difficulty since the gauze structure is very open and allowed the electrical field to form nanofibres. Samples from nanofibres web with viscose nonwoven were taken for SEM imaging and samples from nanofibres webs/gauze were taken for antimicrobial evaluation.

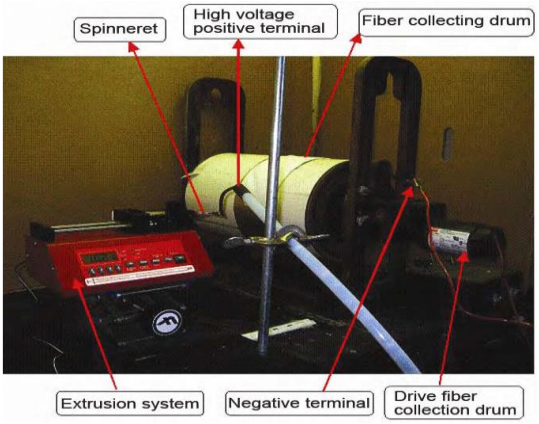

Figure 1: SEM imaging

2.3 Preparation of coating solution

The chitosan was prepared by first dissolving chitosan in acetic acid to yield 1% aqueous acetic acid solution. Then the solution was stirred for 120mins at 60 degree celsius temperature. Then Montmorillonite solution is prepared by mixing powder form in acetic acid. Dissolving Montmorillonite in 1 % aqueous acetic acid solution then it is stirred for 160 mins at 60 degree Celsius temperature. Both the solution are prepared separately and combining that in proper proportion for coating solution. Combination of chitosan and montmorillonite is taken in three forms they are 50/50, 60/40, 70/30 respectively. These solutions are taken for the coating layer on nano film as we prepared in electrospinning.

2.4 Spray coating

Prepared solution are taken in beaker and measured. There is three various of deposition made on the film they are based on the weight by weight criteria. On weight of nano film 5%, 10%, and 15% are taken as various parameters coated on chitosan/PVA blended nano film in three combinations made during solution preparation. At the first empty base material is weighed and after electrospinning again its weighed. The weight difference is the total volume of nano film. In spray coating machine amount of coating solution were taken on film weight basis. Each 10 ml of combined solution contained 0.1 gm of chitosan and montmorillonite. Hence film weight is ranged in 3 gm. The solutions were taken as in content of 6ml, 12ml and

24ml. it is sprayed under normal pressure with 0.1mm dia nozzle. Then it is dried under normal room temperature without ant temperature application.

3. Characterization

The morphology of the nanofibres was analyzed using scanning electron microscope (HITACHI S-3400 SEM) at a voltage of 20 kV. The diameter of the nanofibres was determined at 5 different points. Fourier transform infrared spectroscopy (FTIR- TENSOR 27) was used to identify the change in molecular structure of PVA and chitosan. It was also used to identify the structure of chitosan and PVA blended nanofibres web. The spectra were scanned between 800 cm-1 and 4000 cm-1.

3.1 Antibacterial Activity

The antibacterial activity of nanofibre matrix was determined by disc diffusion method. Nanofibrous matrix having diameter of 35 mm was taken. According to AATCC 147, 1.3 g of nutrient broth was dissolved in 100 mL of distilled water that was taken in the conical flask. The conical flask was plugged with the cotton and then sterilized. After sterilization, slant bacteria was put inside the conical flask and plugged with cotton. The conical flask was kept inside the incubator for 24 h at 37□C. Another conical flask with 100 mL distilled water was taken with 2.4 g of agar and 1.3 g of nutrient broth. This flask was also cotton plugged and sterilized. A negligible quantity of nutrient was poured in the petri plate. A swab stick was taken and dipped in the nutrient that contained bacterial cells. Soon after dipping , it was swabbed in the petri plate that contained the nutrient. The nanofibrous matrix was placed in the prepared petri plate and the antibacterial activity was analyzed from the inhibition zone.

3.2 Blood clotting test

Clotting activity was evaluated as follows
a) Pricked the finger tips using lancet the blood was made to flow continuously.
b) At time of pricking the stopwatch was started simultaneously.
c) Blood drops were blotted after every 30seconds time intervals on one strip at a time. The same procedure was repeated for the remaining two strips and blood clotting time was measured.

3.3 In-Vivo Healing Test

The wound healing characteristic of the chitosan/PVA blended nanofibre was evaluated using two rat models. Two partial thickness wounds of 1cm length were prepared towards the upper side of the organ separately in each rat model. Nanofibre dressing was compared with control wound which was left to the atmosphere to heal. All the experiments with the rat model were preliminary and it was performed with the approval of Institute's Animal ethics committee. Female Wistar rats weighing approximately 180 g were anesthetized by intramuscular injection of Ketamine and Xylaxin, at a dose of 40 mg/kg and 5 mg/kg. The hairs on the skin of the animal were removed and disinfected using 70% ethanol. The wound size was photographed and the length was measured using a scale. The wounds were grossly examined for a period of 5 and 8 days.

4. Results and Discussion

4.1 Electrospinning of Blended Solution 70/30 PVA/Chitosan

PVA was used for blending with chitosan solution to provide spinnability of blended solution and to get better mechanical properties of the fibre. Figure 1 shows SEM photographs of nanofibre web that was obtained from the blended solution of chitosan and PVA. At ratio of 70:30 (PVA/chitosan), The viscosity of the blended solution is one of the perceived parameters that affects the structure and diameter of the fibre. The average diameter of the fibre is found to be 113.6 nm. In these figures shows photograph at various exposures. In fig 2 nano fibres are zoomed in 3000 times of normal view, accordingly 20000 times in fig 2 (b) and 10000 times in fig 2 (c).

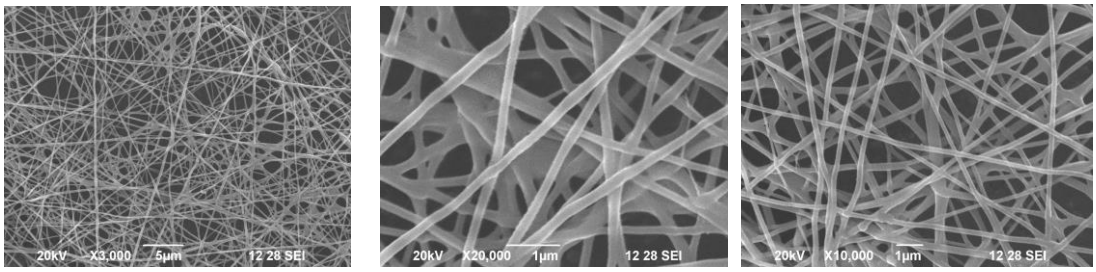

Figure 2: Nano fibers zoomed in: a. 3000 times, b. 2000 times, c. 1000 times

4.2 FTIR Spectral Study

Fourier transform infrared was used to observe peak shift caused due to the interactions between two blended polymers such as hydrogen bonding or any complex structure formation. The FTIR spectra show spectral features similar to those for individual polymers, but some bands shifts from their original positions. Hydrogen bonding is formed between the proton donor and proton acceptor molecules. The intensity of the hydrogen bonding depends upon the acidity of the hydrogen in proton-donor, alkalinity of the proton-acceptor, and distance between the groups. Due to the hydrogen bond formation, the covalent bonds in donor and acceptor become weaker. OH stretching has shifted to lower frequencies compared to pure chitosan. Hydroxyl stretching frequency becomes slightly lower with the increase in chitosan content. It is evident that OH stretching has a favorable extent in changing the molecular spectra.

4.3 Others

As a great achievement, in this study we controlled the heat generation that is among the great concerns associated with the use of montmorillonite. Despite the significant efficacy of montmorillonite in haemostasis, when water is absorbed by the montmorillonite and trapped by hydrogen bond formation, heat is generated. In this light, the induction of exothermic reactions generated from water absorption by inert inorganic elements of montmorillonite (aluminium/silicate), potential burns are major concerns. The heat generated by montmorillonite in contact with aqueous components of the blood in wounds has been reported to reach up to 70 ºC. In our study the maximum temperature rise in the site of the wound was 5 ºC which can be tolerated by the human tissues. As in best combinations derived through various evaluations and final best outcome is treated with rat model. In rat model its survival is calculated after the wound is made. Two male rats were selected and both are wounded through an incision made on it. Then one rat is in controlled manner and another one is treated with PVA/Chitosan nano film with montmorillonite coated. Its blood clotting time and wound healing duration is analysed. It shows effective wound healing with blood clotting function.

5. Conclusion

In this study, PVA/Chitosan nanocomposite fibers were prepared by electrospinning method. The weight ratio of PVA: Chitosan was fixed at 70:30 in1% Acetic acid. The suitable concentration condition of the solution for Chitosan/Montmorillonite deposition is derived 70/30 through drug release evaluation due to the maximum fiber yield. Further more good deposition of coating on nano film was resulted as 15% through bleeding time and blood clotting evaluation hence final product is evaluated in wound healing in a rat model results in Montmorillonite is having good in blood clotting function and combined with that chitosan having higher efficacy in faster rate of wound healing. The result says about Montmorillonite is good in blood clotting but making some restrictions to releasing drug. Hence lower concentration of Montmorillonite gives blood clotting with good rate of wound healing in higher combination of chitosan.

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