Research Article

DETAILED STUDY ON THE BLOOD CLOTTING BEHAVIOUR OF WOUND USING CHITOSAN, SODIUM ALGINATE AND CALCIUM ALGINATE SUBSTRATE

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ABSTRACT

The application of nanoparticle to textile material has been the object of several studies aimed at producing different functional performance. Nanoparticle research is currently an area of intense scientific research, due to a wide variety of potential applications in biomedical, optical, and electronic fields. Lot of research work has been done by using nano fibre technology to find a remedial solution for the problem faced in bio medial field. The outcome research shows that the nano technology can be effectively used in wound care, organ repair. Since the nano fibre got more surface area than the normal material it facilitates more adhesion of cells and drugs. The optimization of process parameters in the electrospinning process, we can get more fibre orientation which results in the enhancement in cell differentiation and drug release control. Biopolymers used in medical applications are alginate, chitosan, hyaluronic acid, collagen, polyurethane, polyethylene oxide, cellulose, gelatin. The production of chitosan nanofiber over polypropylene nonwoven is done by using electrospinning method. Then the coating of chitosan nanoparticle over chitosan nanofiber and a wound dressing material is developed. The effectiveness of the developed wound dressing material is characterized by blood clotting test sodium alginate, calcium alginate, chitosan. Further for characterization of nano particles is done by using FTIR, SEM, particle size distribution technique. From the research work it has been observed that the ball milling process considerably reduces the particle size of components in nano size particle. From SEM analysis we can conclude that after ball milling there is change in morphological structure. Chitosan has good blood clotting function when increasing the concentration of chitosan clotting time is reduced.

Keywords: nano particle, electro spinning, wound dressing, ball milling, chitosan

INTRODUCTION

The application of nanoparticle to textile material has been the object of several studies aimed at producing different functional performance. Nanoparticle research is currently an area of intense scientific research, due to a wide variety of potential applications in biomedical, optical, and electronic fields. Lot of research work has been done by using nano fibre technology to find a remedial solution for the problem faced in bio medial field. The outcome research shows that the nano technology can be effectively used in wound care, organ repair. Since the nano fibre got more surface area than the normal material it facilitates more adhesion of cells and drugs. Second, nanofibers can be fabricated into sophisticated macro-scale structures. The recent work on biomedical nanofibers showed that the large surface area on nanofibers could be translated into enhanced cell activities, drug encapsulation, and drug release rate control. The optimization of process parameters in the electrospinning process, we can get more fibre orientation which results in the enhancement in cell differentiation and drug release control. Biopolymers used in medical applications are alginate, chitosan, hyaluronic acid, collagen, polyurethane, polyethylene oxide, cellulose, gelatin. Biological function of alginate fibre gives strength and flexibility, because of this property it is used as superior wound dressing. Sodium alginate is a biopolymer widely used for wound dressing because of its inherent properties like antibacterial, biocompatibility, absorbing capacity of specific blood solutes and

wound exudates etc. Wound dressing made out of sodium alginate fibre could enhance the positive effect on haemostatic function. In this research work nanoparticle is produced by ball milling technique using sodium alginate, calcium alginate, chitosan biopolymers. The production of chitosan nanofiber over polypropylene nonwoven is done by using electrospinning method. Then the coating of Coating of chitosan nanoparticle over chitosan nanofiber and a wound dressing material is developed. The effectiveness of the developed wound dressing material is characterized by blood clotting test sodium alginate, calcium alginate, chitosan. Further for characterization of nano particles is done by using FTIR, SEM, particle size distribution technique.

Literature Review

The types of diseases and medicine used will decide the blood clotting. If the blood clotting time is more for a patient, it will lead to higher risk of medical treatment. A new emergency haemostat (CX) is being used by the military & other first responders to stop bleeding during major trauma. CX shows great potential to assist clinicians both in speeding normally clotting blood but also in treating bleeding of patients with abnormal clotting ^[1]. Fibres in the scale of nanometres using manmade or natural polymers can be produced using electro spinning. Chitosan, starch and dextran are some of the polysaccharides which can be used for electro spinning. The electro spun nano fibres spun from using this

polysaccharide can be used in the field of tissue engineering and wound dressing.^[2] The biological function of alginates is to give strength and flexibility to the algal tissue and regulate the water content in the seaweed. It is these properties along with the ability to produce fibres from its isomers, which make Alginate the ideal wound dressing. The fibre of an alginate dressing to provide a superior wound-care dressing^[3]. Thrombin is a serine protease that plays a central role in haemostasis by promoting blood coagulation. Thrombin activity under tight regulatory control, and excessive or persistent generation of thrombin has been implicated in a number of respiratory diseases, including acute lung injury, interstitial lung disease, asthma, and chronic lung disease of prematurity.^[4] synthetic polymers and biopolymers have been used to prepare active wound dressings, including polyurethane, chitosan, poly (ethylene oxide)/ poly(vinyl alcohol), alginate, cellulose, and collagen. When such polymeric wound dressings are used for sustained release of therapeutic agents, however, they typically provide limited control of the kinetics with a burst release of drugs within the first few hours^[5]. The gel forming property of alginate helps in removing the

dressing without much trauma, and reduces the pain experienced by the patient during dressing changes^[6]. Chitosan is a natural biopolymer that is derived from chitin, a major component of crustacean outer skeletons. This material is known in the wound management field for its haemostatic properties. Further, it also possesses other biological activities and affect macrophage function that helps in faster wound healing^[7].

ELECTROSPINNING

The electro spinning is a technique where high voltage is used which in turn create a electric charge on the polymer solution. Due to this the solution melts and produces a fibre. For manufacturing the fibre two electrodes namely emitter and collector were used in the spinning machine. Due to surface tension variation by electric field the fluid polymer flows through the

Jet nozzle. The eject3d polymer solution from the jet nozzle under goes a whipping process, then get evaporated and is duly collected by the collector screen.

Figure 1 Electro spinning process (Source <http://www.che.vt.edu>)

A syringe like apparatus is used to hold the polymer solution. The rate of flow of polymer is controlled by a metering pump. Voltage up to 30 KV applied to create electric field. The spinning rate can be controlled by varying the fluid flow and changing the amplitude of electric field.

METHODOLOGY

BLOOD CLOTTING TEST

Before doing an experiment normal blood test is done, to analyze whether the person is in normal condition. Take 3.5ml of blood in a test tube. Now add the biopolymer in different concentration. Tilt the tube 45° to 180° angle for every 30 second. Now the clotting time is calculated using stop watch. Blood sample will change in to cake or gel form. now the time in the stop watch is noted. Blood samples are not exposed to atmosphere. Chitosan, Calcium alginate, sodium alginate both pure and nano particle is tested by this procedure and clotting time is calculated.

ELECTRO SPINNING OF CHITOSAN

The chitosan nanofiber was fabricated using electrospinning from the chitosan using an suitable solvent. 5% of chitosan solution is prepared using an suitable solvent. For electrospinning the resultant solution was loaded with 2 ml plastic syringe and attached to the holder. The needle was connected to the positive output of a high voltage power supply. The collector was wrapped with aluminium foil, grounded, and located at a distance of 10cm from the needle. The flow rate of the solution, applied voltage were fixed to 0.05 ml/min, 20KV respectively. Polypropylene nonwoven material is fixed over aluminium foil and nanofiber is coated over the nonwoven material. Sample is analysed for their morphological structure using scanning electron microscope.

RESULTS AND DISCUSSION NANOPARTICLE SIZE CHARACTERISATION USING PARTICLE SIZE DISTRIBUTION TEST

Particle size distribution is used to analyse the particles from micro meter range to Nano meter range. Using this Particle Size Distribution, particle sizes are analysed for chitosan, sodium alginate, calcium alginate after ball milling

Figure 2 Particle Size Distribution for Sodium Alginate

From the figure 2 we can examine the particle size of sodium alginate after ball milling. From the first peak sodium alginate are in the diameter of 571nm and width of 154nm. From the second peak the particles are in the diameter of 58.06nm and 9.804nm. From the above graph average particle size is 638nm.

Figure 3 Particle Size Distribution for Chitosan

From the above figure 3 we can analyze the particle size of chitosan after ball milling. From the first peak the chitosan particle are in the diameter of 348.7nm and width of 36.72nm. From the above graph average particle size is 969nm.

Figure 4 Particle Size Distribution for Calcium Alginate

From the figure 4, we can analyze the particle size of Calcium alginate after ball milling. From the first peak the calcium alginate are in the diameter of 1568nm and width of 209.2nm. From the above graph average particle size is 2161nm

CHARACTERIZATION OF FUNCTIONAL GROUP BY FTIR ANALYSIS

Using this Fourier Transform Infrared Spectroscopy (FTIR) chemical groups were examined for chitosan, sodium alginate, calcium alginate before and after ball milling to confirm the presence of functional group.

Figure 5 FTIR Test for Sodium Alginate before and after ball milling

From the figure 5 we can compare the FTIR test for sodium alginate before and after ball milling to find the functional group. In sodium alginate before ball milling the following groups are present, peak at 3421.83 cm^{-1} due to presence of OH group. Peak at 2924.18 cm^{-1} due to presence of CH_2 group. Peak at 1604.83 cm^{-1} due to presence of carboxyl group. Peak at 1419.66 cm^{-1} due to presence of carboxyl group. Sodium alginate after ball milling, peak at 3383.26 cm^{-1} due to presence of OH group, peak at 2926.11 cm^{-1} due to presence of CH_2 group, peak at 1600 cm^{-1} due to presence of carboxyl group. peak at 1417.73 cm^{-1} due to presence of carboxyl group.

Figure 6 FTIR Test for Chitosan before and after ball milling

From the figure 6 the FTIR test for Chitosan before and after ball milling can be compared to find the functional groups. In

chitosan before ball milling the following groups are present, peak at 3421.83 cm^{-1} due to presence of OH group. Peak at 2924.18 cm^{-1} due to presence of CH stretching. Peak at 1319 cm^{-1} due to presence of amide II. Peak at 1099.46 and 1020.38 cm^{-1} due to presence of CO stretching. peak at 895 cm^{-1} due to presence of Amide III(NH) group. Chitosan after ball milling, peak at 3419 cm^{-1} due to presence of OH group, peak at 2922 and 2359.02 due to presence of CH stretching group. Peak at 1600.90 cm^{-1} due to presence of Amide II. Peak at 1074 and 1026 cm^{-1} due to presence of CO stretching. Peak at 896 cm^{-1} due to presence of Amide III(NH) group.

Figure 7 FTIR Test for Calcium Alginate before and after ball milling

From figure 7 we can compare the chemical group present after and before ball milling, there is no difference in peak values for the two materials. Before ball milling, the following groups are present, peak at 3418 cm^{-1} due to presence of OH group. Peak at 2929 cm^{-1} due to presence of CH_2 group. Peak at 1604 and 1423 cm^{-1} due to presence of carboxyl group. After ball milling following groups are present, peak at 3412 cm^{-1} due to presence of OH group. Peak at 2933 cm^{-1} due to presence of CH_2 group. Peak at 1604 and 1423 cm^{-1} due to presence of carboxyl group. Peak are present at same place. There is no change in their functional group.

Morphology and particle size characterization using scanning electron microscope (SEM) test

Using this scanning electron microscope (SEM) particle size and morphology were examined for chitosan, sodium alginate, calcium alginate before and after ball milling. The morphological structure of chitosan nano fibre and chitosan nanoparticles coated over chitosan nano fibre were examined using a SEM. The materials were mounted onto sample holder and coated with gold using a sputter coater. SEM images are taken for the above mentioned materials.

Figure 8 SEM images of sodium alginate before and after ball milling

Figure 9 SEM Images of Chitosan Before and After Ball Milling

Figure 10 SEM Images of Calcium Alginate Before and after Ball Milling

Figure 11 SEM images of chitosan nano particle coated over chitosan nanofiber by spray coating method

From the SEM images figure 8 to 10 it can be clearly seen that the particle size of sodium alginate, chitosan and calcium alginate were considerably reduced after ball milling. In case of sodium alginate, the particles are present in the nano meter range (160nm-575nm). After ball milling the particle sizes are reduced in size. Similarly, in case of chitosan the particles are reduced from (36μm-850μm) to (1μm-6μm) which is near to nano meter range after ball milling process. In case of calcium alginate, the particles are reduced from (1.5μ-5μm) to (280nm-650nm) after

ball milling. From this we can come to a conclusion that the ball milling process effectively used to reduce the particle size of a component.

From the figure 11 we can see nanoparticles are coated over nanofiber. So this material can be used as blood clotting wound dressing. Since chitosan nanoparticle has good blood clotting function this material can be used as blood clotting dressings

BLOOD CLOTTING TEST ANALYSIS

Table 1 Blood clotting effect of Raw Biopolymers Added in 0.25 Gram Concentration

Blood	Biopolymer Concentration	Normal blood clotting time before adding biopolymer	Blood clotting time after adding raw biopolymers	Result
3.5 ml	0.25 gram of raw chitoson	8:14 minutes	5 minutes	reduced to 3 minutes 14 seconds
3.5ml	0.25 gram of raw sodium alginate	8:14 minutes	20 minutes	increased to 11 minutes 46 second(used as anticoagulant agent)
3.5ml	0.25 gram of raw calcium alginate	8:14 minutes	11 minutes 10 seconds	increased to 2 minutes 56 second (used as anticoagulant agent)

From the table 1, figure 12, blood clotting time is analyzed for 0.25 gram of biopolymer concentration. Blood clotting time for raw chitoson is reduced to 3 minutes 14 seconds. For raw sodium alginate blood clotting time is increased to 11 minutes 46 seconds,

sodium alginate can be used as anticoagulant agent. for raw sample of calcium alginate blood clotting time is increased to 2 minutes 56 second, so calcium alginate can be used as anticoagulant agent

Figure 12 Effect of Raw Biopolymers on blood clotting at 0.25 Gram Concentration

Table 2 Nano Particle Size Biopolymers are added in 0.25 Gram Concentration

Blood	Biopolymer Concentration	Normal blood clotting time before adding biopolymer	Blood clotting time after adding nano particle size biopolymers	Result
3.5 ml	0.25 gram of nano chitoson	8:14 minutes	4:10 minutes	reduced to 4 minutes 4 seconds
3.5ml	0.25 gram of nano calcium alginate	8:14 minutes	10 minutes	increased to 4 minutes 46 second(used as anticoagulant agent)
3.5ml	0.25 gram of nano sodium alginate	8:14 minutes	18 minutes	increased to 9 minutes 46 second(used as anticoagulant agent)

From the table 2, figure 13, blood clotting time is analyzed for 0.25 gram of nano particle size biopolymer concentration. Blood clotting time for nano chitoson is reduced to 4 minutes 4 seconds. For nano calcium alginate blood clotting time is increased to 1

minute 46 seconds, so calcium alginate can be used as anticoagulant agent. for nano sodium alginate blood clotting time is increased to 9 minutes 46 second, so sodium alginate can be used as anticoagulant agent

Figure 13. Effect of Nano Particle Size Biopolymers on blood clotting at 0.25 Gram Concentration

Table 3 Raw Biopolymers are added in 0.1 Gram Concentration

Blood	Biopolymer Concentration	Normal blood clotting time before adding biopolymer	Blood clotting time after adding raw biopolymers	Result
3.5 ml	0.1 gram of raw chitosan	8:14 minutes	8 minutes	reduced to 0.14seconds
3.5ml	0.1 gram of raw sodium alginate	8:14 minutes	10 minutes	increased to 1 minutes 46 second(used as anticoagulant agent)
3.5ml	0.1 gram of raw calcium alginate	8:14 minutes	10 minutes	increased to 1 minutes 46 second (used as anticoagulant agent)

From the table 3, figure 14 blood clotting time is analyzed for 0.1 gram of raw biopolymer concentration. Blood clotting time for raw chitosan is reduced to 0.14 seconds. For raw sodium alginate blood clotting time is increased to 1 minute 46 second, so sodium

alginate can be used as anticoagulant agent. for raw sodium alginate blood clotting time is increased to 1 minutes 46 second, so calcium alginate can be used as anticoagulant agent

Figure 14 Effect of Raw Biopolymers on blood clotting at 0.1 Gram Concentration

Table 4 Nano Particle Size Biopolymers are added in 0.1 Gram Concentration

Blood	Biopolymer Concentration	Normal blood clotting time before adding biopolymer	Blood clotting time after adding nano particle size biopolymers	Result
3.5 ml	0.1 gram of nano chitosan	8:14 minutes	8 minutes	reduced to 0.14seconds
3.5ml	0.1 gram of nano sodium alginate	8:14 minutes	10:30 minutes	increased to 2 minutes 16 second(used as anticoagulant)
3.5ml	0.1 gram of nano calcium alginate	8:14 minutes	8:30 minutes	increased to 0.16 second (used as anticoagulant agent)

From the Table 4. figure 15 blood clotting time is analyzed for 0.25 gram of nano particle size biopolymer concentration. Blood clotting time for nano chitosan is reduced to 0.14 seconds. For nano sodium alginate blood clotting time is increased to 2 minutes

16 seconds, so sodium alginate can be used as anticoagulant agent. For raw sodium alginate blood clotting time is increased to 0.16 second, so calcium alginate can be used as anticoagulant agent.

Figure 15 Effect of Nano Particle Size Biopolymers on blood clotting at 0.1 Gram Concentration

Figure 16 Comparison of Raw and Nano Biopolymers on blood clotting at 0.25 and 0.1 Gram Concentration

From figure 16 when comparing the raw and nano biopolymers, clotting time is reduced for nano sized particle. Among three biopolymers chitosan has good blood clotting function when compared to calcium alginate and sodium alginate. For chitosan when concentration of is increased the clotting time is reduced.

When concentration is reduced clotting time is reduced. Similarly, when concentration of calcium alginate and sodium alginate is increased blood clotting time is increased from normal clotting time, when concentration is reduced clotting time is reduced when comparing with 0.25g concentration test result.

CONCLUSION

From the three biopolymers chitosan, calcium alginate, sodium alginate nanoparticle characterization was examined. The average particle size can be modified by ball milling process. From SEM analysis, It can be concluded that after ball milling there is change in morphological structure. Chitosan has good blood clotting function when increasing the concentration of chitosan, clotting time is reduced. When concentration for sodium alginate and calcium alginate is reduced clotting time is reduced. When concentration is increased clotting time is increased. When comparing raw and nano biopolymers, nano particle sized biopolymer has taken less clotting time when compared to raw biopolymers. Chitosan nanoparticle is coated over chitosan nano fibre. This wound dressing material can be effectively used as blood clotting dressings.

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