Research Article [Parthasarathi & Thilagavathi, 2(4): April, 2011]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Development of hemostatic wound dressing using silk

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Abstract

Defence field and various other pharmacutical areas are facing the major problem of traumatic hemorrhage. Hemostatic wound dressings help control traumatic external bleeding by enhancing or accelerating the natural clotting process through various physical reactions. The fatal traumatic hemorrhage remains one of the most challenging problems for military people and other pharma areas .Hence the development of effective biocompatible hemostatic wound dressings that overcome these limitations is an absolute necessity. The hemostatic wound dressing with characteristics such as toxicity, biodegradability and less cost has to be developed. The goal of this research work is to develop hemostatic wound dressing with silk fibre for controlling the traumatic external bleeding. First, the silk fibers were treated with two kinds of neutral salts calcium nitrate tetra-hydrate ($Ca(NO_3)_2 4H_2O$) and calcium chloride systems in order to decrystallize their crystalline structure and improve their water absorbability and biodegradability. FTIR and X ray demonstrated that most effective decrystallization of silk fibers were performed with the treatment in $Ca(NO_3)_2 4H_2O$. Next, the blood clotting ability of the treated silk fibers was investigated by blood coagulation test.

Key-Words: Bleeding wound dressing, Hemostatsis, Silk dressing, Biodegradable silk

Introduction

Hemostasis is a physiological process which always maintains the integrity of the vascular system by immediately causing the arrest of bleeding from injured blood vessels. It is majorly responsible for minimizing blood loss from the damaged vessels[1]. The most challenging problems for both military and civilian medicine is the fatal traumatic hemorrhage. Currently, it has been reported that uncontrolled hemorrhage causes almost 50% of battlefield deaths and 80% of civilian trauma deaths in India. This leads to the need of development of effective hemostatic dressings that help control traumatic internal and external bleeding, like topical hemostatic wound dressings[2-3]. The topical hemostatic wound dressings work by enhancing or accelerating the natural clotting process through various physical reactions between the dressings and blood. The ideal Hemostatic dressings are required to have high hemostatic action, as well as biodegradability, ease of sterilization, low cost performance, and can be tailored to specific needs.

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However, many commercially available hemostatic wound dressings have some disadvantages, such as side effects, lack of biodegradability, potential for bacterial infections, high cost performance, and hardness of the material. For example, zeolites may cause major thermal injuries which come from the exothermic reaction, remain as foreign bodies in open wounds, and are toxic to the eyes or lungs[4-5].

Material and Methods

The materials used for the study were 100% degummed Bombyx Mori silk. Raw silk was degummed prior to experiments. Sericin was removed with 0.25 % (w/v) sodium lauryl sulphate and 0.25% (w/v) sodium carbonate in boiling water, bath ratio of 1:100(w/v), for 1 hour. After the degumming, fibroin was washed in boiling water for 1 hour to remove remaining sericin and surfactants and then washed again with distilled water.

Decrystallisation of silk fibre

Decrystallisation of silk fibre was done with two neutral salts. Calcium Nitrate Tetra Hydrate was one of the neutral salts. The other salt used for decrystallisation was calcium chloride.

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Preparation of solvent system

Calcium nitrate tetra-hydrate [$Ca(NO_3)_2$ $4H_2O$ was dissolved in methanol at 70 deg temperature to prepare solutions with concentrations of 25% (w/w) and 50% (w/w). In the same manner, calcium chloride [$CaCl_2$] was dissolved in methanol with concentration of 25% (w/w), and 50% (w/w).

Decrystallisation was done by using weight method at the concentration of 25% and 50%.The temperature was kept for about 60-75 degree centigrade for 2 hours.

Calcium Nitrate Tetra Hydrate

First the chemicals and samples were taken on the weight basis method. A corresponding concentration of water and methonal was taken in a beaker. Then 25% concentration of chemicals were added and stirred well. Then the samples were loaded into the beaker without any entanglements. The same method was followed for the 50 % high as that of 25% concentration.

Table1: Parameters of solvent

Chemicals	Conc. (%)	Temperature (degree)	Time (hour)
Calcium Nitrate Tetra Hydrate	25%	60 - 75	2
	50%		
Calcium Chloride	25%	60 - 75	2
	50%		

Calcium chloride

As per the weight basis method the samples and chemicals were taken. A beaker was taken, and then certain concentration of water and methonal was added. Then 25 % concentrations of chemicals were added and stirred well. Then the samples were loaded into the beaker without any entanglements. The same method was followed for the 50 % concentration as that of 25% concentration.

Characterisation of silk fibre

FTIR

FTIR spectra of all samples were obtained using FTIR Spectrometer with software. Accessory was used for the spectra of the degummed silk fibers and treated fibers in solvents. All scans were performed with an average of 20 repeated scans from 4000 to 400 cm^{-1}

FTIR Test (Fourier Transmission Infrared Reflectance) was conducted in order to find whether the treated sample is characterised or not. Characterisation means surface modification of the fibre. Normally the silk fibre is crystalline in nature, in order to make it amorphous we carried out the decrystallisation process. The FTIR test was taken to find the amount of amorphous region covered in the given sample. The reflectance principle was used .It was already set for one minute 20 scans.

Scanning electron microscopy (SEM)

Various silk fibers were examined by the scanning electron microscope (SEM). The samples were sputter-coated with gold in order to minimize electron charging on the surface and to obtain fine images. Their surface morphology was examined.

Blood coagulation test

The Blood Coagulation test was done to find how much time/seconds was taken to clot the blood. For this test, we had taken sheep blood as it contains high plasma content. First the sheep blood was kept aside for some time to make the plasma to come up to the surface. The plasma was taken with the help of the syringe and poured it into the test tube. Then a pinch of treated sample was loaded into the test tube with the help of the glass rod. As soon as the sample touched the blood the stop watch was switched on. Then it was noted, how much time it took to clot the blood. Accordingly this process was repeated for all the samples of lower and higher concentrations

Results and Conclusion[3-5]

Decrystillisation of silk fibre

Decrystillisation process was mainly carried out to change the surface characteristics of silk fibre. Calcium nitrate tetra hydrate and calcium chloride were used to make the modifications.

Calcium Chloride

Two beakers were taken, in which samples and chemicals were taken in 2 concentrations 25% and 50% on the basis of weight method. Then these were loaded into the hot water bath at a temperature of 60-75 degree centigrade for two hours.

Calcium Nitrate Tetra Hydrate

Two beakers were taken, in which samples and chemicals were taken in 2 concentrations 25% and 50% on the basis of weight method. Then these were loaded into the hot water bath at a temperature of 60-75 degree centigrade for two hours.

Charaterization of silk using FTIR and SEM

FTIR

Charaterisation of the silk fibre was studied by, FTIR. The FTIR spectrum of the degummed silk fibers confirmed the presence of sheet conformation at each amide peak. Treated silk fibers in different concentration of the solutions showed almost the same profiles as that of the degummed silk in the spectra, exhibiting the absorption bands assigned to β-sheet crystalline structure at around 1655 cm-1 (amide I), 1545 cm-1 (amide II), and 1265 cm-1 (amide III), respectively.

ISSN: 0976-7126

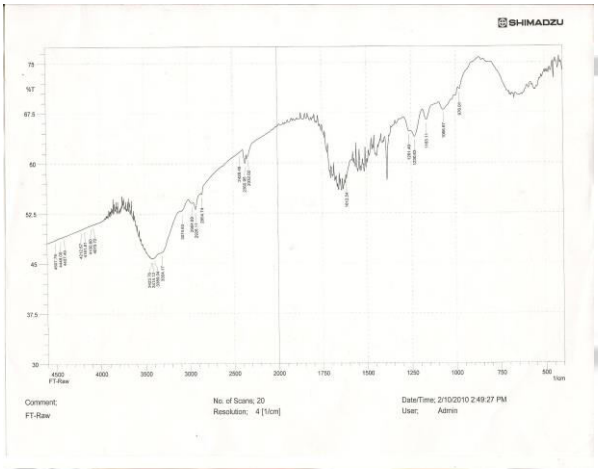

Fig. 1: FTIR of Untreated Silk Fibre

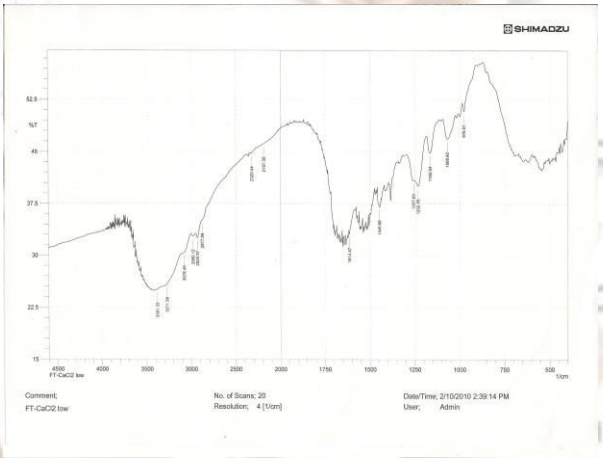

Fig. 2: FTIR of 25% Calcium Chloride

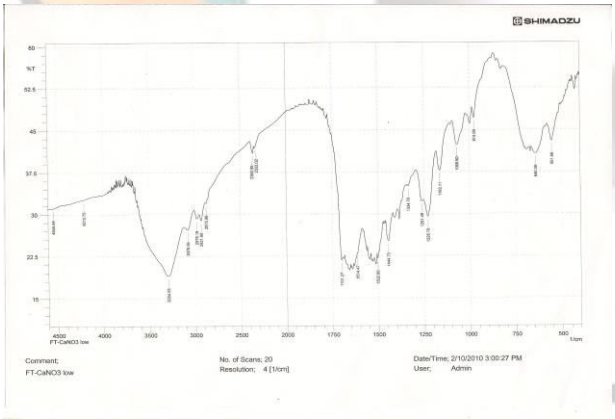

Fig. 3: FTIR of 25% Calcium Nitrate Tetra Hydrate

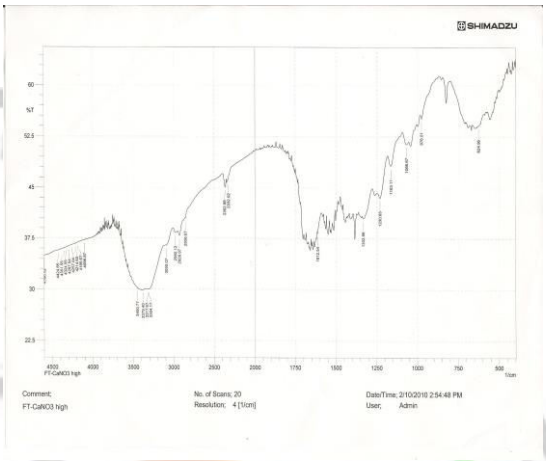

Fig. 4: FTIR of 50% Calcium Nitrate Tetra Hydrate

SEM images of the degummed silk fibers and the treated silk fibers in 50% (w/w) $Ca(NO_3)_2$ $4H_2O$/MeOH at 70 degee temperature are shown below. The degummed silk fibers exhibited relatively uniform diameter with smooth surface. The fibers treated in 50% (w/w) $Ca(NO_3)_2$ $4H_2O$/MeOH dissolved partially. As there was an increase in solution concentration of $Ca(NO_3)_2$ $4H_2O$/MeOH, the silk fibers seemed gradually to dissolve from their outside, resulting in the reduction in the diameter. The cracking of the fiber surface was also observed, for the fibers treated in higher concentration solutions. The fibers treated in 50% (w/w) at 65 deg temperature visibly looked different, from the degummed silk fibers and other treated fibers. These fibers were easily broken by tweezers. We found that the solvent easily penetrated inside the fibers and caused the severe decrystallization both outside and inside under this condition.As a result, the fibres started to dissolve and stuck to each other. The tensile properties demonstrated that the flexibility was lost while stiffness increased for these fibers.

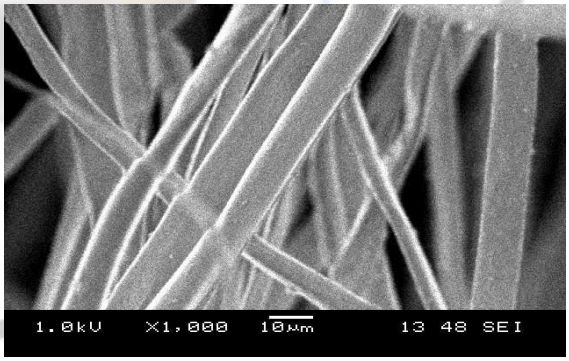

Fig. 5: SEM Image of Untreated silk fibre.

ISSN: 0976-7126

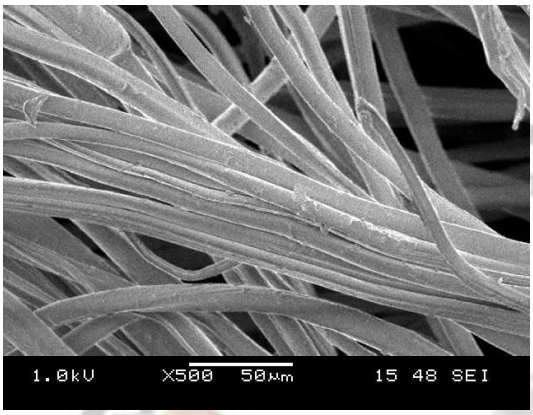

Fig. 6: SEM Image of 25% $CaCl_2$

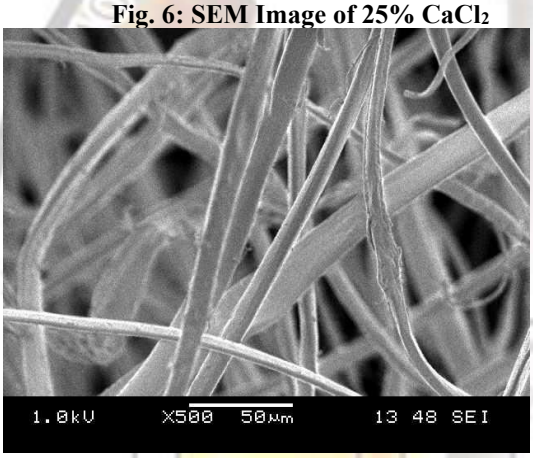

Fig. 7: SEM Image of 50% $CaCl_2$

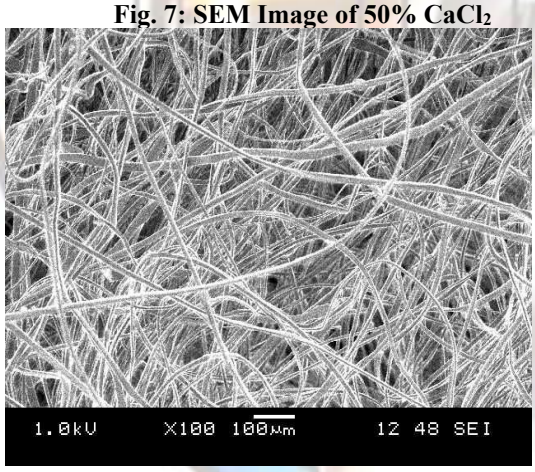

Fig. 8: SEM Image of 25% $Ca(NO_3)_2$ $4H_2O$

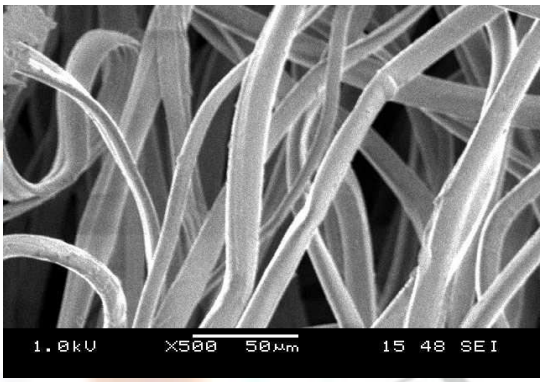

Fig. 9: SEM Image of 50% $Ca(NO_3)_2$ $4H_2O$

Blood Coagulation Test

The blood coagulation test was done to compare which treated sample was more absorbant and took less time to clot the blood.

Table 2: Blood Coagulation Test Result

Chemicals	Conc. (%)	Time (sec.)
Control Sample	-	40
Calcium Chloride	25	32
	50	15
Calcium Nitrate Tetra Hydrate	25	42
	50	9

The original silk fibres were decrystallized with two kinds of salt Calcium Chloride and Calcium Nitrate Tetra Hydrate with Methanol and water. The chemical structure of the treated silk fibers was characterized by FTIR and SEM Analysis to investigate the degree of decrystallization. Any obvious shifts of specific peaks that correspond with conformational transition from sheet to random coil, amide I, II, III, were not observed in FTIR spectra, even though there was some change in peak intensity. We could not exactly confirm whether decrystallization was achieved or not, through FTIR data. Since FTIR spectra are usually considered as qualitative analysis, other quantitative techniques must be needed to determine the amount of crystalline structure. In this study FTIR method was used, to get more accurate data about the crystallinity.

As the decrystallization proceeds, the fibers initially absorbed more water; however, the amount of absorbed water was small after equilibrium was reached. We consider these results that the increased amorphous region due to the decrystallization brought about the increase in amount of absorbed water initially. The blood clotting ability of the treated silk fibers was investigated by blood coagulation test. Calcium nitrate Tetrahydrate thereby exhibits good blood coagulation property

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