

A novel wound dressing material — fibrin–chitosan–sodium alginate composite sheet

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MS received 1 December 2011; revised 23 February 2012

Abstract. The present study describes preparation and characterization of fibrin–chitosan–sodium alginate composite (F–C–SA) in sheet form. F–C–SA composite was prepared and characterized for its physicochemical properties like water absorption capacity, surface morphology, FTIR spectra and mechanical properties. The optimum quantities of fibrin, chitosan and sodium alginate to get better mechanical properties to composite were determined. FTIR spectrum confirmed the interaction between amino groups of chitosan, fibrin and sodium alginate and SEM studies revealed composite nature of the material.

Keywords. Chitosan; fibrin; sodium alginate.

1. Introduction

Wound dressing based on alginic material is well known in literature as well as from commercial point of view, in wound management (Paul and Sharma 2004). Alginate based dressings are used as dressing materials for bleeding wounds as they exhibit hemostatic properties (Amy *et al* 2009). The gel forming property of alginate helps in removing the dressing without much trauma and reduces the pain experienced by the patient during the change of dressing. It also provides a moist environment that leads to rapid granulation and re-epithelization. Wound dressing materials like hydrogels, films, hydrocolloids and foams are also being used for haemostatic purposes. Specialized additives with special functions can be introduced in advanced wound dressings with the aim to absorb odours, provide strong antibacterial properties, sooth pain and relieve irritation (Jing *et al* 2009). The naturally occurring biomaterials include various forms of types I and II collagen-based biomaterials, in the form of scaffold matrices (Speer *et al* 1979; Sams and Nixon 1995; Frenkel *et al* 1997; Nehrer *et al* 1997), gels (Kimura *et al* 1984; Wakitani *et al* 1989; Kang *et al* 1997) or collagen–alginate composite gels (Qi and Scully 1997).

Chitin and chitosan are described as a family of linear polysaccharides consisting of varying amounts of β -(1–4) linked residues of *N*-acetyl-2-amino-2-deoxy-*D*-glucose (glucosamine) and 2-amino-2-deoxy-*D*-glucose (*N*-acetyl-glucosamine) residues. Chitosan is a natural polymer composed of randomly distributed β -(1–4)-linked

D-glucosamine (deacetylated unit) and *N*-acetyl-*D*-glucosamine (acetylated unit). In acidic conditions, even fully protonated chitosan tends to form aggregates as a result of hydrogen bonds and hydrophobic interactions. This hydrophobic behaviour is based on the presence of both the main polysaccharide backbone and the *N*-acetyl groups at C2 position (Rinaudo 2006). The strong functionality of chitosan (two hydroxyl groups (C3, C6) and one primary amine group (C2) per-repeat unit) gives it a considerable opportunity of chemical modification. Several reviews covering the chemistry of chitosan have been recently published (Alves and Manoa 2008; Mourya and Inamdar 2008).

Chitosan is known in the wound management field for its haemostatic properties. Further, it also possesses other biological activities and affects macrophage function that helps in faster wound healing (Balassa and Prudden 1984). It also has an aptitude to stimulate cell proliferation and histoarchitectural tissue organization. The biological properties including bacteriostatic and fungistatic properties are particularly useful for wound treatment (Muzzarelli 1989).

Fibrin, a blood plasma protein, is a minor component which is essential for clot formation, even a 60–100 mg/ml of fibrin can initiate coagulation, hampering blood-flow. Fibrin glue or fibrin sealant is also referred to as a fibrin based scaffold and used to control surgical bleeding, speed wound healing, seal off hollow body organs or cover holes made by standard sutures, and provide slow-release delivery of medications like antibiotics to tissues exposed (Atrah 1994). Fibrin scaffold use is helpful in repairing injuries to the urinary tract (Andrew 1987), liver (Feinstein 2001), lung (Bastarache 2009), spleen (Modi and Rahamim 2005), kidney (Patel 2003) and heart (Toda *et al* 2007).

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Sodium alginate has applications as a material for the encapsulation and immobilization of a variety of cell types for immunosolatory and biochemical processing applications. It forms a biodegradable gel when crosslinked with calcium ions and it has been exploited in cartilage tissue engineering since chondrocytes do not de-differentiate when immobilized in it (Wang *et al* 2003). The combination of alginate and chitosan via ionic interaction between carboxylate moieties on alginate and protonated amines on chitosan to form polyelectrolyte complex (PEC) in the form of membrane (Lishan *et al* 2002), capsule (Gaserod *et al* 1998), fibre and scaffold (Li *et al* 2005) has been investigated widely. The chitosan–alginate PEC membrane was prepared as a wound dressing via gradually mixing alginate solution and chitosan solution in the presence of acetone (Wang *et al* 2002). It was shown in SEM results that the chitosan–alginate composite scaffold has an interconnected, homogeneous porous structure (Li *et al* 2005).

It was reported that a dressing with an optimal combination of chitosan, alginate and polyethylene glycol with a synergistic combination of an antibiotic and an analgesic was studied on human subjects with chronic non-healing ulcers. It was observed that this material made the ulcer cleaner and had beneficial effect in the control of infection (Shelma 2008). There are reports suggesting that certain alginate dressings (e.g. Kaltostat) can enhance wound healing by the stimulation of human monocytes to produce elevated levels of tumour necrosis factors such as α -interleukin-6. The production of these cytokines at the wound site results in a pro-inflammatory stimulus advantageous to wound healing to a great extent. The high level of bioactivity of these dressings is believed to be due to the presence of endotoxin in alginates (Thomas *et al* 2000). The carboxylate moieties on alginate can ionically interact with the protonated amines on chitosan, forming physical cross-linked hydro-gels known as polyelectrolyte complex (PEC) (Takahashi *et al* 1990; Augst *et al* 2006). Compared with the constituent polymers, PEC reduces tendency of swelling and improves structural strength and mechanical stability (Dang and Leong 2008) and there has been considerable interest in the chitosan–alginate PEC systems used in tissue engineering in the form of scaffolds (Baruch and Machluf 2006; Lim *et al* 2008) membranes (Takahashi *et al* 1990), fibres and microcapsules (Li *et al* 2005). Many observations have revealed about cell growth in chitosan and sodium alginate scaffolds using cell imaging techniques and transplantation of the scaffolds into rats.

In order to create a moist environment for rapid wound healing, a hydrogel sheet composed of a blended powder of alginate, chitin/chitosan and fucoidan has been developed as a functional wound dressing. The desirable degradation rate of chitosan–alginate scaffolds can be achieved through adjusting the ratio of chitosan/alginate to suit the need of biodegradable medical engineering. In addition, alginate wound dressings also have novel hemostatic and antimicrobial properties as well as the ability to promote wound healing. They are now widely used in the management of

highly exuding wounds such as leg ulcers, pressure sores and surgical wounds (Yimin 2008).

The rheological behaviour of chitosan, sodium alginate and their blends at several compositions (chitosan–sodium alginate, 2/8, 4/6, 5/5, 6/4, 8/2) has been studied (Natarajan *et al* 2005). Composites, in film form, containing physiologically clotted fibrin, chitosan and gelatin (FCG) were prepared and crosslinked with glutaraldehyde. The amount of individual constituents, which gave maximum tensile strength to the FCG composite, was optimized. Polyols like glycerol, ethylene glycol and poly ethylene glycol gives flexibility to the composite materials (Diana *et al* 1999). In this present study, PEG is used as a plasticizer.

In this study, a composite was prepared containing fibrin, chitosan and sodium alginate (F–C–SA) and characterized for its physicochemical properties like water absorption capacity, surface morphology, FTIR spectra and mechanical properties.

2. Materials and methods

2.1 Purification of crude fibrin (F)

Physiologically clotted fibrin was purified as described elsewhere (Ravindra Babu *et al* 1989). Fresh bovine blood was collected from the nearby slaughter-house and churned with a glass rod to isolate crude fibrin. Later the crude fibrin was washed thoroughly under running water to remove blood clots. The washed fibrin was treated with 0.5 M sodium acetate solution and 20% hydrogen peroxide solution. The fibrin was then washed thoroughly under running water and ground to a paste in a mixer. This paste contained 40% solids.

2.2 Preparation of chitosan from crab shells (C)

Chitosan was prepared by the modification of earlier method (Mochizuki *et al* 1989). The crab shells were cleaned thoroughly with water to remove sand and impurities. Then the shells were treated with 3% sodium hydroxide solution for about 5 h for de-proteinization. The product was washed well with water and treated with hydrochloric acid solution (2 N) to remove calcium carbonate. The resultant product was chitin. The pure chitin thus obtained was treated with 40% sodium hydroxide and heated in a sand bath maintained at about 200°C for 2 h. The contents were routinely checked for their solubility in hydrochloric acid (0.1 N) solution. Once the contents were dissolved in hydrochloric acid, the same was removed from the bath, washed thoroughly with water and dried well. The resultant product, chitosan, was characterized and degree of deacetylation and molecular weight was found to be 85% and 5.1×10^5 g mol⁻¹, respectively. 2% Chitosan solution was prepared by dissolving 2 g of chitosan in 100 ml of 0.1 N HCl for further experiments.

2.3 Preparation of sodium alginate solution (SA)

Sodium alginate powder was obtained from Sigma, Aldrich Co. USA. 1% Sodium alginate was prepared by dissolving in warm water, followed by heating at 70 °C, while stirring until the solution became clear.

2.4 Preparation of fibrin–chitosan composite (F–C composites)

FC composites were prepared by mixing different stoichiometric ratios of fibrin paste and chitosan solutions. 1% polyethylene glycol was added to each composition. The details are given in table 1. The solution was stirred for 3 h to obtain homogenous solution and poured into petridishes and dried at 40°C. The dried sheets were put in polythene covers and stored for further use.

2.5 Preparation of fibrin, chitosan and sodium alginate composite (F–C–SA)

Various stoichiometric proportions of fibrin paste and chitosan solutions were mixed thoroughly using a stirrer. 1% Poly ethylene glycol was added to each composition. The details are given in table 1. The stoichiometric ratio which gave better mechanical properties was used for the preparation of fibrin, chitosan and sodium alginate composite (in this case sample containing 4 w/v % fibrin and 0.1 w/v % chitosan was taken). Keeping the fibrin (4 w/v %) and

chitosan (0.1 w/v %) as constant ratio, amount of sodium alginate solution was varied. 1% Poly ethylene glycol was added to each composition. The details of film are given in table 2. These solutions were stirred well and cast into films in the petridishes previously washed with ethanol having a diameter of 10 cm and dried at room temperature.

3. Characterization

3.1 Mechanical properties

Two dumb bell shaped specimens of 4 mm width and 10 mm length were punched out from the films prepared. The mechanical properties such as tensile strength and percentage strain at break were measured using an Instron 4501 tensile system according to Vogel (1971) at an extension rate of 10 cm/min. The force and elongation were measured when the film broke off. The values are the average of 3 experiments. The tensile strength and elongation at break were calculated as shown in (1) and (2).

$$\begin{aligned} \text{Tensile strength(N/mm}^2) & \\ &= \text{breaking force (N)/cross-} \\ &\quad \text{sectional area of sample (mm}^2), \end{aligned}$$

$$\begin{aligned} \text{Elongation at break (\%)} & \\ &= \frac{\text{Increase in length at breaking point (mm)}}{\text{Initial length (mm)}} \times 100. \end{aligned}$$

Table 1. Mechanical properties of fibrin and chitosan composites.

Sample no.	Fibrin (w/v %)	Chitosan (w/v %)	Thickness	% Elongation	Tensile strength (MPa)
1.	4	0	Unable to form film		
2.	0	0.2	0.16 ± 0.01	8.90 ± 0.71	10.12 ± 0.07
3.	4	0.06	0.23 ± 0.02	12.50 ± 0.23	16.52 ± 0.03
4.	4	0.1	0.26 ± 0.01	12.50 ± 0.14	18.82 ± 0.29
5.	4	0.2	0.27 ± 0.03	5.28 ± 0.36	17.60 ± 0.06
6.	4	0.3	0.29 ± 0.01	5.44 ± 0.34	14.56 ± 1.03
7.	4	0.4	0.33 ± 0.01	4.44 ± 0.19	13.78 ± 1.07

Mean and S.D. values were calculated from seven different samples.

Table 2. Mechanical properties of fibrin, chitosan and sodium alginate composites.

Sample no.	Fibrin (w/v %)	Chitosan (w/v %)	Sodium alginate (w/v %)	Thickness	% Elongation	Tensile strength (MPa)
1.	4	0.1	0.05	0.28 ± 0.15	12.50 ± 0.23	13.58 ± 0.62
2.	4	0.1	0.1	0.31 ± 0.01	5.28 ± 0.14	17.60 ± 0.35
3.	4	0.1	0.15	0.33 ± 0.02	5.44 ± 0.36	22.18 ± 0.24
4.	4	0.1	0.2	0.35 ± 0.13	4.44 ± 0.34	23.34 ± 1.04
5.	4	0.1	0.25	0.42 ± 0.14	5.02 ± 0.28	19.89 ± 0.18

Fibrin solution, 40% in water, chitosan solution, 2% in 0.3 N acetic acid, PVP solution, 5% in water.

The thickness of the films at 5 different locations (centre and 4 corners) was measured with a digital micrometer (QLR digit, IP4, Qinghai, China) and mean thickness was calculated. The values are the average of 3 experiments.

3.2 Water absorption capacity of films

Estimation of water absorption capacity was done by the method explained by Rao *et al* (2007). The WAC of fibrin, chitosan, chitosan–fibrin composites and chitosan, fibrin and sodium alginate composites were determined by swelling small pieces of each sample of known weight in distilled water at room temperature. The swollen weights of the samples were determined by first blotting the samples with filter paper followed by accurately weighing the sample. The weights of the swollen pieces were recorded every 1 h, 2 h, 3 h and after 24 h. Percentage swelling of the samples at a given time was calculated from the formula:

$$ES = \frac{W_s - W_0}{W_0} \times 100,$$

where W_s is the weight of the sample (moist) at a given time, W_0 the initial weight of the sample and ES the percentage of swelling at a given time.

3.3 Fourier transform infrared spectroscopy

Fourier-transform IR (FT–IR) studies of samples were carried out using Nicolet Impact 400 FT–IR spectroscopy. The Nicolet Impact 400 FT–IR is a very high end optical bench with a range of 7400–375 cm^{-1} . The frequency range is 400–5000 cm^{-1} , the spectral resolution is 0.125 cm^{-1} and the beam splitter is KBr (375–7000 cm^{-1}).

3.4 Scanning electron microscopy

The prepared films were mounted on metal grids with double-sided adhesive tape, coated with gold to $\sim 500 \times 10^{-8}$ cm in thickness using SC7640 sputter coater (Quorum Technologies, Newhaven, UK) under high vacuum, 0.1 Torr, 1.2 kV and 50 mA at $25^\circ\text{C} \pm 1^\circ\text{C}$. The surface morphology of coated samples was examined by scanning electron microscopy (SEM; JEOL JSM-5200, Tokyo, Japan) at 20 kV.

3.5 Statistical analysis

In vitro data obtained from each experiment were subjected to statistical analysis using 1-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparisons test. Differences between the groups were tested for significance by the χ^2 test for the *in vivo* studies. Significance was indicated by $P < 0.05$. In addition, film thickness was highly increased by enhancing the chitosan and sodium alginate concentration in the formulation (A1–A3; $P < 0.05$; tables 1 and 2).

4. Results and discussion

4.1 Strength and porosity

The tensile test provides an indication of the strength and elasticity of the film, which can be reflected by tensile strength and elongation at break. It is suggested that films suitable for wound dressing should be preferably strong but flexible.

During the past few years, significant research has been carried out in the field of biological dressings with the aim of improving the wound healing properties of these materials. In the present study, a biocomposite sheet was prepared containing fibrin, chitosan and sodium alginate. All the three biomaterials are well known for their biomedical properties. Chitosan as such do not have enough mechanical strength required for a wound dressing material, however, it has antimicrobial property as well as wound healing capacity. To give better tensile strength to the end product, fibrin and sodium alginate were added to the composite (Natarajan *et al* 2005). Poly ethylene glycol was added to give flexibility to the composite.

In the first series of experiments, F–C composites were prepared with various stoichiometric ratios (table 1). Sample 4 containing 4 w/v % of fibrin and 0.1 w/v % chitosan has given better mechanical property compared to other composites. This sample was used for further studies and for the preparation of fibrin–chitosan–sodium alginate composites. Among the various stoichiometric ratios used to prepare F–C–SA, the composition containing 4 w/v % fibrin, 0.1 w/v % chitosan and 0.2 w/v % sodium alginate (sample 4) gave better mechanical properties compared to the other composites (tensile strength, –23.54/elongation, 4.4%). Hence, sample 4 was used for further characterization. Fibrin as such formed a discontinuous film and was brittle and hence the films could not be prepared. Even though chitosan solution formed a film after drying, it has exhibited comparatively lower mechanical strength.

The tensile strength of the samples increased up to sample 4 and later decreasing trend was observed. This may be due to the increasing quantity of chitosan. The elongation percentage of sample 4 was also found to be good compared to other samples. It can be observed from table 1 that there is an increase in the tensile strength of F–C–SA composites up to sample 4 and later decreasing trend is observed. Except for sample 1, there was not much difference in the values of elongation percentage. Based on these mechanical studies, sample 4 from table 2 was taken for further analysis.

4.2 FTIR

FT–IR spectrum of fibrin, chitosan and sodium alginate and F–C–SA are given in figure 1. As the fibrin is a protein, it has shown the characteristics amide absorption bands at 1550 cm^{-1} and 1240 cm^{-1} representing amides II and III groups (figure 1b). Figure 1a shows FT–IR of chitosan, the

following absorption bands, 2364 cm^{-1} representing C–N asymmetric band stretching, 1653 cm^{-1} representing amide-I band and C–O stretch of acetyl group, 1375 cm^{-1} showing asymmetric C–H bending of CH_2 group and 1071 cm^{-1} showing skeletal vibration involving bridge C–O stretch. The characteristic peaks of alginate appeared at 3429 , 1630 and 1428 cm^{-1} , corresponding to hydroxyl (OH), carbonyl (C=O) and carboxyl (COOH), respectively. The FT-IR spectrum of F–C–SA is given in figure 1d. However, the peaks are shifted, the carboxyl groups of alginate are shifted from 1428 to 1414 cm^{-1} . The amide-I peak of fibrin and carbonyl peak of sodium alginate was merged and shifted to 1615 cm^{-1} . Amides-II and III peaks representing fibrin are absent. The carboxyl group of alginate might have reacted with protonated NH_2 groups on chitosan and fibrin, forming a

physical cross linking known as polyelectrolyte complex (Augst *et al* 2006). The shifts are due to the formation of the complex.

4.3 Water absorption capacity

The water absorption capacity of a biomaterial is an important factor when it is applied onto an open wound surface. A biomaterial with better water absorbing properties absorbs wound exudates and keeps the wound dry, and thereby prevents air borne infection. In the present study, the water absorption capacities of F–C–SA composites prepared at different time intervals are given in table 3. In the prepared composites, sample 4 exhibited better water absorption

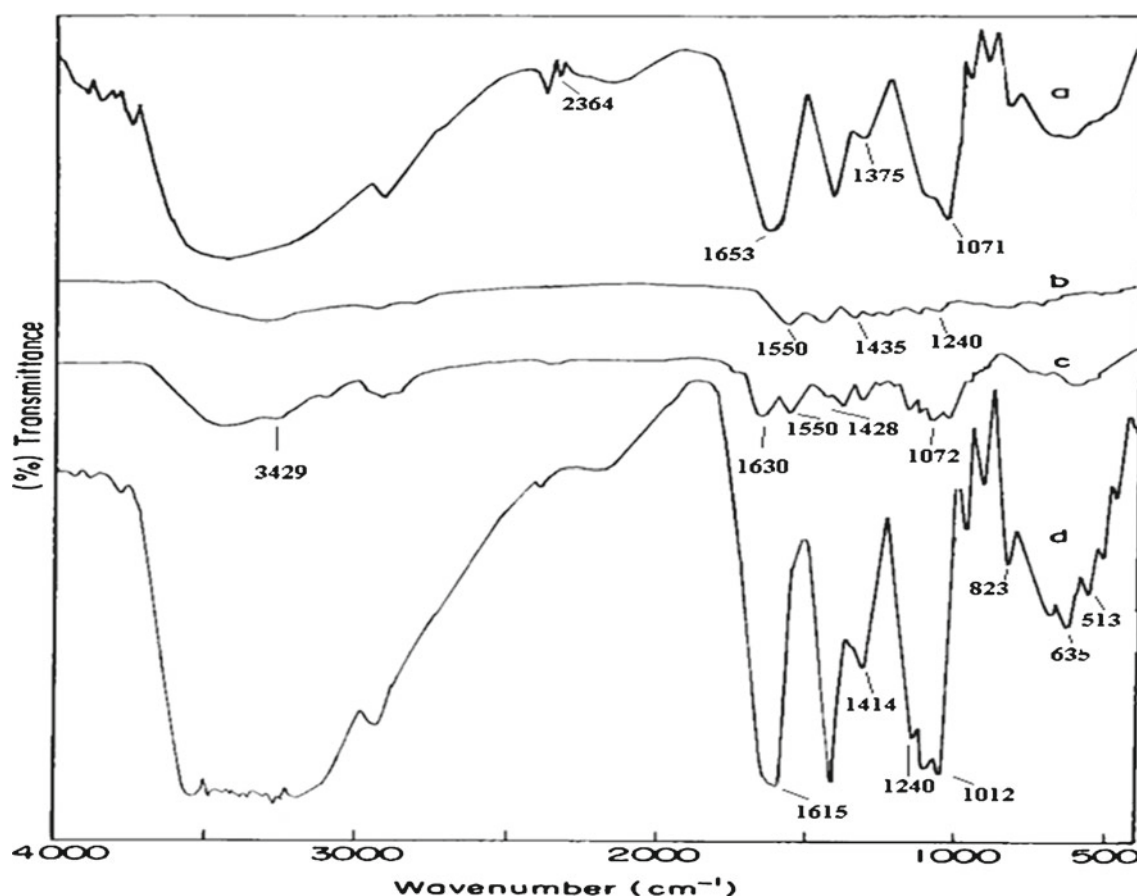


Figure 1. FTIR spectra of chitosan (a), fibrin (b) and sodium alginate (c) and fibrin chitosan and sodium alginate composite sheet (d).

Table 3. Water absorption capacity analysis for F–C–SA.

Sl. No.	Time	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1.	After 1 h	$69\% \pm 9.13$	$77\% \pm 10.35$	$86\% \pm 12.65$	$94\% \pm 10.78$	102 ± 10.91
2.	After 2 h	$81\% \pm 10.81$	$92\% \pm 11.42$	$99\% \pm 11.36$	$105\% \pm 9.76$	113 ± 0.47
3.	After 3 h	$101\% \pm 10.76$	$108\% \pm 11.24$	$116\% \pm 10.03$	$121\% \pm 11.82$	130 ± 0.51
4.	After 24 h	$103\% \pm 13.81$	$109\% \pm 11.51$	$118\% \pm 10.65$	$132\% \pm 10.16$	Dissolved

Data are expressed as a mean of three experiments.

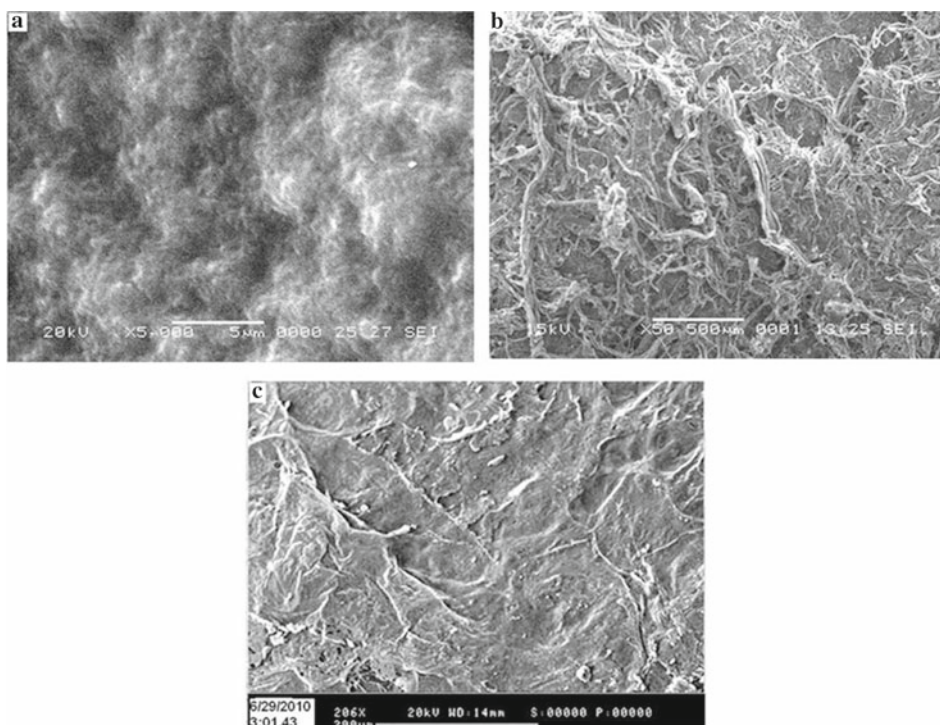


Figure 2. SEM picture of surface of C-SA composite (a), fibrin alone (b) and F-C-SA composite (c).

capacities at various time intervals compared to all the other composites. However, samples 1, 2 and 3 also have shown more or less similar water absorption capacities as sample 4. These types of wound dressing materials are supposed to help in hastening the wound healing process compared to conventional materials.

4.4 SEM analysis

SEM pictures of C-SA, fibrin alone (F) and F-C-SA composite are given in figure 2. C-SA composite film (figure 2a) shows plain surface with porous nature, composite nature of the film is seen. The surface morphology of fibrin (figure 2b) shows fibrous nature. SEM picture of F-C-SA composite (2c) shows reduction in the fibrous nature in the surface and pores are also observed. The reduction in fibrin nature is due to the presence of chitosan and sodium alginate. The porous nature of the matrix helps in absorbing wound fluids effectively and thereby keeping wound dry. This eventually helps in the prevention of bacterial infection and quicker healing. The composite is highly porous with a pore size around 100–400 μm , a structure favourable for cell attachment and skin formation.

5. Conclusions

A natural polymer-based complex scaffold was prepared that has improved mechanical properties when compared with its chitosan counterpart and is structurally stable due to

the strong ionic bonding between the amine groups of chitosan and the carboxyl groups of alginate. By applying right biomaterial with the required porous size, surface morphology, high strength can indeed provide a good composite for wound dressing applications. F-C-SA composite with compositions of sample 4 in table 3 was selected and applied on the clinical wounds of dogs to find its efficacy as wound dressing material and the study is in progress.

References

- Alves N M and Manoa J F 2008 *Int. J. Biol. Macromol.* **43** 401
- Amy C, Daniel H and Rosemary W 2009 *Technology* 1
- Andrew L 1987 *J. Urol.* **169** 1360
- Atrah H I 1994 *BMJ* **308** 933
- Augst A D, Kong H J and Mooney D J 2006 *J. Macromol. Biosci.* **6** 623
- Balassa L L and Prudden J F 1984 *Application of chitin and chitosan in wound healing acceleration, in Chitin, chitosan and related enzymes* (San Diego: Academic Press) pp 296–305
- Baruch L and Machluf M 2006 *Biopolymers* **82** 570
- Bastarache J A 2009 *Am. J. Physiol. Lung Cell Mol. Physiol.* L275
- Dang J M and Leong K W 2008 *J. Drug Target.* **16** 437
- Diana C W S, Carola H, Allan J E and Ralph P C 1999 *J. Agric. Food Chem.* **47** 3432
- Feinstein A J 2001 *J. Biol. Med.* **74** 315
- Frenkel S R, Toolan B, Menche D and Pitman M 1997 *J. Bone Joint Surg.* **79** 831
- Gaserod O, Smidsrod O and Skjak-Braek G 1998 *Biomaterials* **19** 1815

- Jing H, Ziyou Z, Ruixue Y, Dongzhi Y and Jun N 2009 *Int. J. Biol. Macromol.* **11** 25
- Kang R, Marui T, Nita I, Hi G, Suh J-K and Robbins R D 1997 *OA Cartilage* **5** 139
- Kimura T, Ysui N, Ohswara S and Ono K 1984 *J. Clin. Orthop. Relat. Res.* **186** 231
- Li Z, Ramay H R, Hauch K D, Xiao D and Zhang M 2005 *Biomaterials* **26** 3919
- Lim S M, Song D K, Oh S H and Lee-Yoon D S 2008 *J. Biomater. Sci. Polym. Ed.* **19** 453
- Lishan W, Eugene K, Aileen W and Lee Yong L 2002 *J. Biomed. Mater. Res. Part B* **63** 610
- Mochizuki A, Sato Y, Ogawara H and Yamashita S 1989 *J. Appl. Polym. Sci.* **37** 3357
- Modi P and Rahamim J 2005 *Eur. J. Cardiothorac. Surg.* **28** 167
- Mourya V K and Inamdar N N 2008 *React. Funct. Polym.* **68** 1013
- Muzzarelli R A A 1989 *Applied science* (London: Elsevier) p. 87
- Natarajan N, Shashirekha V, Noorjahan S E, Kumar Ramesh, Rose C and Sastry T P 2005 *J. Macromol. Sci. Part A* **42** 945
- Nehrer S, Breinan H A, Ramappa A, Shortkoff S, Young G, Minas T, Sledge C B and Yanna I V 1997 *J. Biomed. Mater. Res.* **38** 95
- Patel R 2003 *J. Endourol.* **17** 799
- Paul W and Sharma C P 2004 *Trends. Biomaterial. Artif. Organ.* **18** 18
- Qi W N and Scully S P 1997 *J. Orthop. Res.* **15** 483
- Rao K P, Joseph K T and Nayudamma Y 2007 *J. Polym. Sci. A Polym.* **9** 3199
- Ravindra Babu P, Sastry T P, Rose C and Muralidhara Rao N 1989 *J. Appl. Polym. Sci.* **37** 3357
- Rinaudo M 2006 *J. Macromol. Biosci.* **6** 590
- Sams A E and Nixon A J 1995 *OA Cartilage* **3** 47
- Shelma 2008 *Trends Biomaterial. Artif. Organ.* **22** 111
- Speer D P, Chvapil M, Volz R G and Holmes M D 1979 *J. Clin. Orthop. Relat. Res.* **144** 326
- Takahashi T, Takayama K, Machida Y and Nagai T 1990 *Int. J. Pharm.* **61** 35
- Thomas A, Harding K G and Moore K 2000 *J. Biomater.* **21** 1797
- Toda K, Yoshitatsu M, Izutani H and Ihara K 2007 *J. Interact. CardioVasc. Thorac. Surg.* **6** 577
- Vogel H G 1971 *Biochem. Biophys. Acta* **252** 580
- Wakitani S, Kimura T and Hirroka A 1989 *J. Bone Joint Surg.* **71** 74
- Wang L, Khor E, Wee A and Lim L Y 2002 *J. Biomed. Mater. Res.* **63** 610
- Wang L, Shelton R M, Cooper P R, Lawson M, Triffitt J T and Barralet J E 2003 *J. Biomater.* **24** 3475
- Yimin Q 2008 *Polym. Int.* **57** 171