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Fibrin–Chitosan–Gelatin Composite Film: Preparation and Characterization

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Composites, in film form, containing physiologically clotted fibrin, chitosan and gelatin were prepared and crosslinked with glutaraldehyde. The films were characterised for their IR spectroscopy, water absorption capacity (WAC) at different pH conditions, mechanical properties and scanning electron microscopy (SEM). Fibrin + gelatin films gave higher WAC values in all the pH ranges (2,7 and 10) studied. However, with the addition of chitosan, the WAC values of the composite decreased. This was attributed to the crosslinking of glutaraldehyde with the hydrophilic groups available on chitosan and gelatin/fibrin. The amount of individual constituents, which gave maximum tensile strength to the FCG composite, was optimized. SEM pictures of the FCG have exhibited the fibrous and porous nature of the composite.

Keywords physiologically clotted fibrin, chitosan, gelatin, hydrogel, biocompatible

Introduction

Biomaterials are macromolecules, which are prepared from biological origins and used for various clinical and industrial purposes. For widespread application, a biomaterial should have the following essential characteristics; it should be non-irritant, non-toxic, non-antigenic, non-carcinogenic, sterilizable and adequately available. Fibrin, chitosan and gelatin seem to offer most of these characteristics to serve as biomaterials.

Fibrin is a good haemostatic and wound dressing material that can be made in the form of a sponge, film, powder, sheet, etc. Bergel (1) observed that fibrin powder spread on wounds not only helped blood clotting but also promoted wound healing. Ferry and Morrison (2, 3) were the first to describe the preparation of fibrin film from human plasma. They prepared the film by dissolving Cohn fraction I of human plasma at a pH of 6.2–6.4 to give a solution containing 0.5% fibrinogen and about 0.4% other plasma proteins. This solution was mixed with thrombin solution and was poured into a rectangular dish where it was left to clot for about 1 h. Depending on the fibrinogen concentration the thickness of the film thus obtained may vary from 0.03–2 mm. Matras et al. (4), described the successful application of a fibrin glue in peripheral nerve repair in rabbits and the procedure was later used in nerve anastomoses in humans (5). Gibble and Ness (6) found that a fibrin sealant was effective in providing haemostasis, sealing vascular

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anastomoses and promoting wound healing in humans. In the present study, crude fibrin, which is until now discarded as a waste in most of the Indian slaughter houses, is used as a raw material for the preparation of pure fibrin. Sastry et al. (7), used this material for the preparation of hydrogels based on fibrin–gelatin composites. As fibrin does not form a continuous film, gelatin was used as a reinforcement material in their study. These hydrogels have exhibited improved physical properties like water absorption capacity, tensile strength and thermal stability.

Chitosan and its derivatives possess many advantageous properties that make them especially interesting materials. They are non-toxic and can be biodegraded into normal body constituents. Chitosan is an unbranched polymer with a high charge density in solution and carries a positive charge at pH below 6.5. Many uses of chitosan are based on its "+ve charge" which is attracted to "-ve charge" materials. Most living tissues like skin, bone and hair are negatively charged and therefore chitosan can be used to remove toxic and contaminating bio-burden materials such as protein and heavy metals for safety reasons (8, 9). Chitosan possesses haemostatic, fungistatic, anticancer and anti-chlesteremic activities. Chitosan accelerates the tensile strength of wounds by speeding the fibroblastic synthesis of collagen in the initial phase of wound healing (10). Chitosan could achieve haemostasis and promote normal tissue regeneration (11) and it was also found that it exhibits bacteriostatic and fungistatic properties (12).

Gelatin is a natural polymer used in pharmaceutical and medical applications, especially in the production of biocompatible and biodegradable wound dressings and drug delivery systems (13–18). Gelatin granules hydrate, swell and solubilize in water and rapidly degrade *in vivo*. The durability of these materials could however, be prolonged by crosslinking with aldehydes, carbodiimides, and aldose sugars. Recently, uses of gelatin based biomaterials in applications of artificial skin and neuron regeneration have been reported (19). The interest in gelatin arises mainly from the fact that these natural polymers allow the production of biocompatible and biodegradable biomaterials, which can act as temporary replacements (20).

In the present article fibrin–chitosan–gelatin (FCG) composite was prepared in a sheet form. As fibrin alone could not form a continuous film, gelatin was added to the fibrin. Chitosan acted as a binder and had advantageous properties as discussed earlier. Glutaraldehyde was used as crosslinking agent to give better shelf life and ethylene glycol was used as plasticizing agent to give flexibility to the FCG composite.

Experimental

Materials

Fibrin was purified from the crude fibrin available at a local slaughterhouse as described earlier (2). The purified fibrin contains 60% solids and denoted as 'F'. Gelatin was purchased from MBD gelatins, Mumbai, India and chitosan was supplied by Central Fisheries Research Institute, Tuticorin, India. All the other reagents used were of analytical grade.

Methods

Preparation of Chitosan Solution (C). 0.5 g of chitosan was dissolved in 100 mL of 0.3 N HCl.

Preparation of Gelatin Solution (G). 10 g gelatin was dissolved in 100 mL of water at 55°C in a water bath.

Preparation of FCG Composite. Fibrin paste and chitosan and gelatin solutions were mixed in different stoichiometric ratios as described in Table I. Ethylene glycol and glutaraldehyde were added as plasticizing and crosslinking agents respectively. This mixture was poured into polythene trays (measurements 12 cm × 7.5 cm) and dried at room temperature (30°C) to get FCG films.

Characterization

Infrared Spectroscopy

The infrared (IR) spectra of FCG composite was taken in a Nicolet Impact 400 Fourier Transform Infrared Spectrometer, using a 500 mg KBr pellet containing 2–6 mg of the sample.

Water Absorption Capacity

Water absorption capacity (WAC) of the films prepared was estimated at different pH conditions (2, 7, 10), by a method described elsewhere (22). The WACs of the films were determined by swelling small pieces of each sample of known weight in distilled water for pH 7; in dilute sodium carbonate solution for pH 10; in dilute solution of HCl for pH 2 at room temperature. The swollen weights of the samples were determined by first blotting the samples with filter paper, and then accurately weighing the sample. The weights of the swollen pieces were recorded every 1, 2 and 3 and after 24 h. Percentage swelling of the samples at a given time was calculated from the formula

$$E_s = \frac{W_s - W_o}{W_o} \times 100$$

where W_s is the weight of the sample (moist) at a given time, W_o is the initial weight of the sample and E_s is the percent of swelling at a given time.

Tensile Strength

Two dumbbell-shaped specimens, 4 mm wide and 10 mm long were punched out of the prepared films. Mechanical properties such as tensile strength and percentage of strain

Table 1
Water absorption capacity of FCG films at pH 2

Film	Fibrin	Gelatin	Chitosan	1 hr	2 hrs	3 hrs	24 hrs
1	25	5	0	350.98	358.62	372.54	370.41
2	25	5	2	333.75	347.50	365.25	361.00
3	25	5	5	249.19	276.61	279.83	280.16
4	25	5	10	188.54	210.41	217.70	220.83
5	25	0	5	269.64	267.85	269.64	271.42
6	25	0	0	329.47	341.05	362.10	365.26

at break were measured using an Instron 4501 tensile testing system (23) at an extension rate of 5 mm/min.

Scanning Electron Microscopy

Scanning electron micrographs were taken for FCG using a JSM 5300 Scanning Microscope.

Results and Discussion

The major drawback for the biomaterials containing proteins or polysaccharides is their quick solubility. In order to reduce the solubility and retain the biomaterial for longer time *in vivo*, crosslinking agents like glutaraldehyde and genipin are being used. In this study, glutaraldehyde is used as the crosslinking agent to impart stability and mechanical strength to FCG composites.

Infrared Spectroscopy

The FTIR spectrum of FCG composite (Figure 1) contains all the characteristic absorption peaks of fibrin, chitosan and gelatin. As fibrin and gelatin are proteins, IR spectrum exhibits the amide absorption bands at around 1652 cm^{-1}, 1539 cm^{-1} and 1241 cm^{-1}. The marginal shift in these absorption bands (originally at 1660 cm^{-1}, 1550 cm^{-1} and 1240 cm^{-1}) may be attributed to the inter- molecular crosslinking between –CHO groups available on the backbones of gelatin and fibrin and free NH_2 group of the chitosan molecule. This may also be explained as the Schiff base reaction between –CHO groups

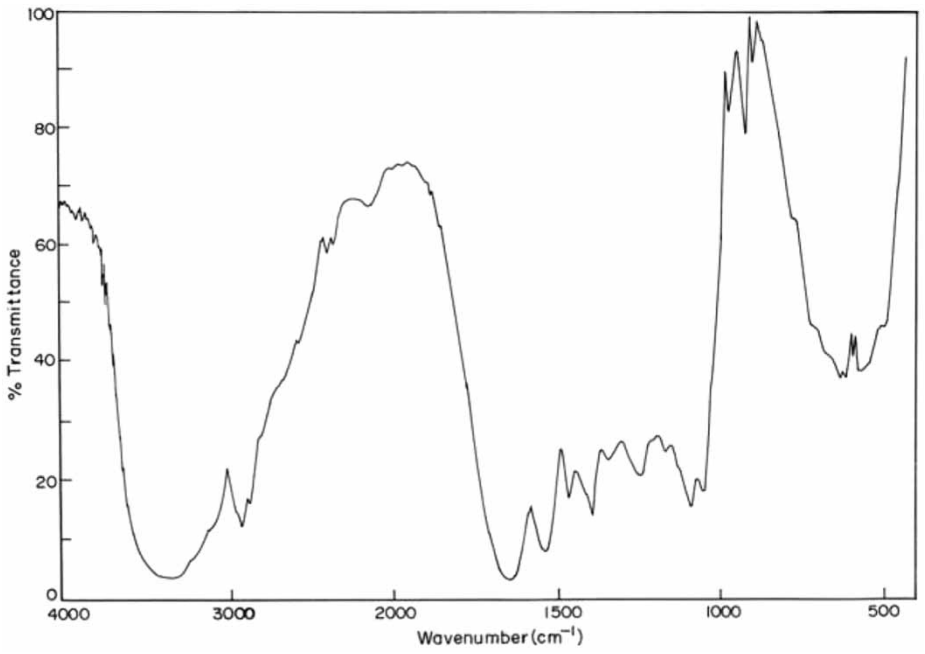

Figure 1. FTIR Spectrum of FCG composite.

of glutaraldehyde and free NH_2 groups of proteins and chitosan. The peak at 1384 cm^{-1} represents the –C–O stretching of primary alcoholic group ($-CH_2-OH$) in chitosan. A broad band in the 3400–3200 cm^{-1} range represents the hydrogen bonded –OH in the composite.

Water Absorption Capacity

WACs of biomaterials are very important when they are used as wound dressing materials. These materials absorb the wound exudates and keep the wound dry and prevent airborne infection. In this direction, water absorption studies of FCG composite were done at different pH conditions and are described in Tables 1, 2, 3.

Film No. 1 gave higher WAC values when compared to other films in all 3 pH ranges studied. As fibrin and gelatin are proteineous in nature and have – OH, NH_2 and COOH groups on their backbones, it is natural that their WAC values are higher. But with an increase in the amount of chitosan solution in the FCG, the WAC values decreased. This can be explained as follows: The two –CHO groups of glutaraldehyde might have crosslinked with the $-NH_2$ groups of both fibrin/gelatin and chitosan thereby forming a bridge (Schiff bases) between the two backbones as shown in Figure 2.

Film No. 4 has shown lesser WAC values as the amount of chitosan was doubled in these samples. However, WAC values of the FCG composites were higher in acidic and alkaline pH ranges when compared to those at neutral pH ranges. This is because the fibrin is the major constituent in FCG and it does not absorb water as effectively as it does at alkaline or acidic pH. Similarly, FCG Film No. 6 (without chitosan) gave higher WAC values when compared to Film No. 5 and here also the free NH_2 groups on the chitosan would have reacted and blocked some of the hydrophilic groups on fibrin and gelatin.

Tensile Strength

Mechanical properties of the biomaterials in general, as wound covering materials in particular, are very important, as they have to be handled by physician while applying them onto wound surfaces. Keeping this important point in view, we have studied the tensile properties of the films prepared (Table 4). FCG Film No. 6 (Fibrin alone) exhibited very poor tensile strength when compared to other fibers. However, the values were improved with addition of the gelatin and chitosan. With the increase in the amount of

Table 2
Water absorption capacity of FCG films at pH 7

Film	Fibrin	Gelatin	Chitosan	1 hr	2 hrs	3 hrs	24 hrs
1	25	5	0	150.81	186.88	189.34	189.01
2	25	5	2	139.49	147.89	152.94	159.24
3	25	5	5	124.20	154.52	155.94	152.35
4	25	5	10	120.80	178.00	137.08	137.20
5	25	0	5	125.19	152.40	152.32	152.52
6	25	0	0	135.74	159.84	160.08	160.52

Table 3
Water absorption capacity of FCG films at pH 10

Film	Fibrin	Gelatin	Chitosan	1 hr	2 hrs	3 hrs	24 hrs
1	25	5	0	255.43	301.08	319.56	315.78
2	25	5	2	157.14	216.88	259.74	263.63
3	25	5	5	215.92	243.13	254.86	250.48
4	25	5	10	204.65	212.79	216.97	216.04
5	25	0	5	180.70	188.59	196.49	151.75
6	25	0	0	281.65	293.57	297.24	280.73

chitosan solution from 2 mL to 5 mL in the FCG composite, the tensile strength was increased and when the addition of chitosan solution was doubled (10 mL) the tensile values have shown a steep fall. This may be explained as the free $-NH_2$ groups on chitosan might have reacted with most of the functional groups on the fibrin/gelatin

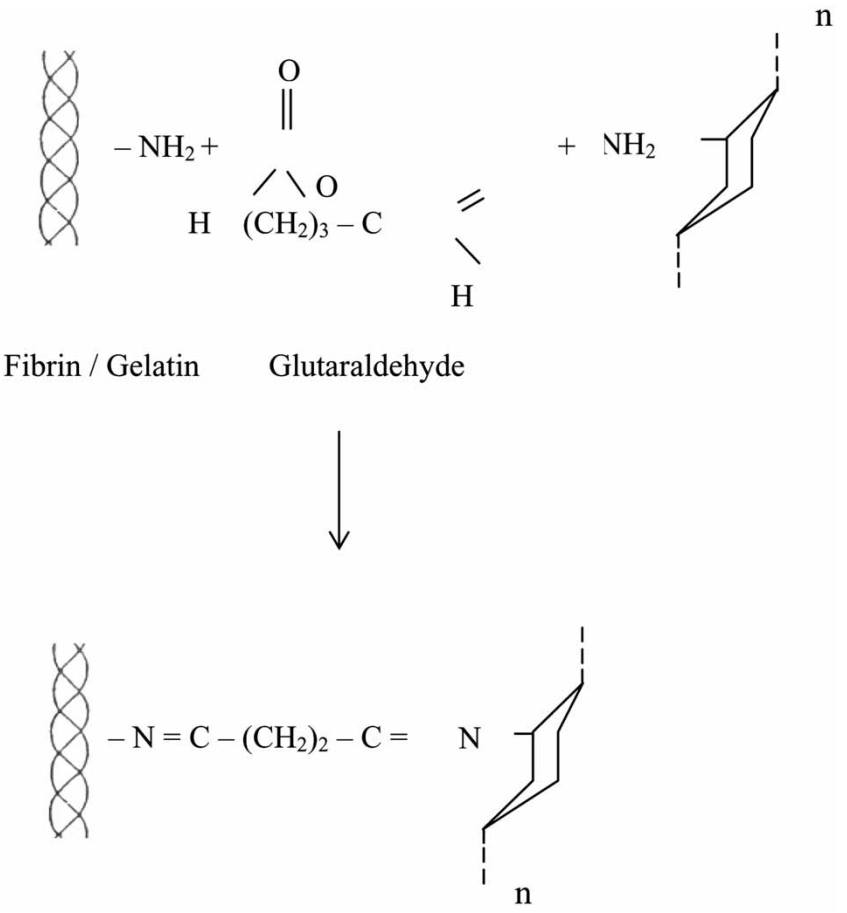

Figure 2. Schiff base formation.

Table 4
Mechanical properties of FCG composite films

Film	Fibrin paste (60%) solids	Gelatin	Chitosan	Tensile strength	% of elongation at break
1	25	5	0	3.21	15
2	25	5	2	5.7	15
3	25	5	5	9.5	57
4	25	5	10	3.5	36
5	25	0	5	3.9	299
6	25	0	0	0.6	149

through glutaraldehyde and thereby causing brittleness in the composite. Film No. 3 gave better tensile values when compared to other films.

Scanning Electron Microscopy

The SEM picture of FCG taken at lower magnification (Figure 3) has shown beautiful fibrous nature on the surface of the film. Whereas the photomicrograph taken at higher magnification (Figure 4) has shown not only the fibrous nature on the surface but also its porous nature. This property is an added advantage for the composite as the wound exudates can evaporate through the pores and oxygen supply for the wound is enhanced.

Conclusion

FCG composites were prepared and characterized for their physico-chemical properties. The IR spectrumof FCG composite has shown all the characteristics absorption bands

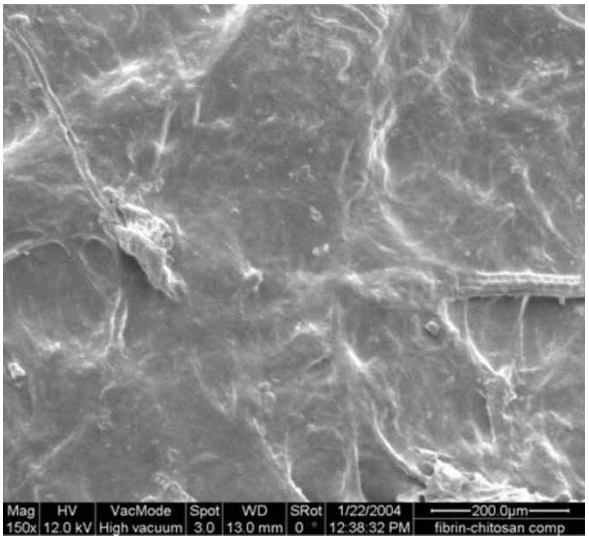

Figure 3. SEM picture of FCG at 150 X magnification.

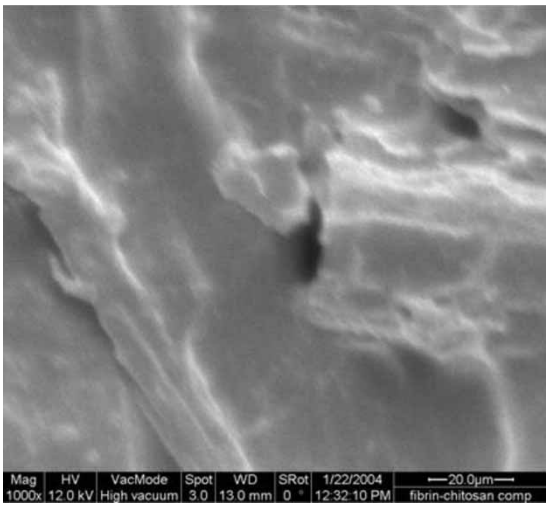

Figure 4. SEM picture of FCG at 1000 X magnification.

of fibrin, chitosan, and gelatin. Water absorption capacity values of the neutral pH ranges. Film No. 3, which contains 25 g fibrin paste, 5 mL gelatin (5%) and 5 mL chitosan solution (0.5%), gave better tensile values when compared to other films. SEM pictures have shown fibrous and porous nature of the FCG composites.

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