Hydrogels Based on Gelatin Poly(hydroxyethyl methacrylate) and Poly(butyl acrylate) Graft Copolymer Impregnated with Fibrin

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ABSTRACT: Gelatin was graft copolymerized with poly(hydroxyethyl methacrylate and butyl acrylate) and subsequently crosslinked with glutaraldehyde. Fibrin of bovine origin was incorporated onto this graft copolymer and characterized for the percentage of grafting. The infrared spectroscopy, mechanical strength, and water absorption capacity of the composite were also studied. This biomaterial can be used as a hemostat in many phases of surgery and as a wound dressing material. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **65:** 555–560, 1997

Key words: graft copolymerization; gelatin; fibrin; hydroxyethyl methacrylate; butyl acrylate

INTRODUCTION

The concept of temporary biological dressings, introduced in the 1930s, has been developed to the point where their use has been extended from burns to many types of granulating wounds. Although recent introduction of topical antibiotics has allowed a greater percentage of burns to proceed to healing without grafting, the need for wound closure by autografts remains incomplete. In 50% full-thickness burns of an adult, the amount of skin required to obtain wound closure has been estimated as 6000 cm^2.[1] This large amount of donor area is not always available on the patient, therefore other biomaterials like synthetic or natural polymers and their composites have been sought for temporary closure of the wound.

Fibrin has good hemostatic and wound healing properties and it can be made in the form of sponge, film, powder, fibrin glue, etc.[2–5] Fibrinogen has been isolated from whole blood using centrifugation in combination with cryoprecipitation, ethanol, ammonium sulfate, or polyethyleneglycol precipitation. Concentrated solutions of fibrinogen, when resolubilized and mixed with thrombin, are used as fibrin glue or a fibrin adhesive to adhere tissues together or as a fibrin sealant to close tissue defects in many phases of surgery.[6] In the local municipal slaughterhouse, fibrin is isolated daily from 1000 L of blood by churning the blood with a stirrer, and the defibrinated blood is collected by a few pharmaceutical companies to isolate biochemicals like hemoglobin. The fibrin is wasted or sold at a cheaper rate to be used as a fertilizer for plants. This fibrin is used in this article along with the graft-copolymerized gelatin as a hemostatic/wound dressing material.

Gelatin is obtained by partial hydrolysis of collagen derived from skin, white connective tissue, and bones of animals.[7] Gelatin is well known for its wound healing properties.[8–10] Gelatin can also be chemically modified so that it can function as a better wound dressing material. An absorbant powder that will protect the wounds from bacte-

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rial contamination is prepared by modifying gelatin with acrylic monomers.[11] Gelatin graft poly(methacrylate) for use as artificial skin was prepared by Min, Lee, and Kim.[12] Gelatin was graft-copolymerized with different acrylic polymers by various authors for different purposes.[13–16]

In this article gelatin is graft-copolymerized with poly(hydroxyethyl methacrylate) and poly(butyl acrylate) and fibrin is added to the grafted gelatin. Grafting of the selected polymers onto the gelatin will improve the functionality and hydrophilicity of the products and it can be handled easily while applying onto the wound. It will behave like skin and will adhere to the contours of the wound properly.

EXPERIMENTAL

Materials

Materials used were gelatin [Pharmaceutical grade (MBD gelatin, Bombay)], fibrin (purified from the crude fibrin available at a local slaughterhouse, Madras), hydroxyethyl methacrylate (HEMA), (Fluka, Switzerland), and butyl acrylate (Fluka, Switzerland). All other reagents used were of analytical grade.

Methods

Purification of Fibrin

The fibrin collected from the slaughterhouse was in crude form containing blood clots. It was washed thoroughly under running water to remove the blood clots, and further treated with 0.5*M* sodium acetate solution to remove the remaining blood stains. The resultant material was bleached with 20 mL hydrogen peroxide solution (30% v/v) per liter at pH 8.0 (pH was adjusted with 0.1 *N* sodium hydroxide solution). This bleached fibrin was removed from the bleaching bath, washed thoroughly with cold running water, and ground to pulp by using a mixer. The ground mass was cast to form film. It may be stored as a film or in the form of a powder. The film form of the fibrin is denoted as "F" in the text.

Preparation of Gelatin Film

Ten g of gelatin was dissolved in 100 mL of water at 55°C in a water bath. The solution was poured into a polythene tray, spread uniformly and dried at 40°C in a hot air oven. The gelatin film obtained was removed and stored in a polythene cover. It is denoted as "G."

Preparation of Gelatin–Fibrin Film

Ten g of gelatin was dissolved in 100 mL of water at 55°C in a water bath. To this, 0.25 gm of fibrin was added and mixed thoroughly. Then the contents were poured into a polythene tray, spread uniformly and dried at 40°C in a hot air oven. The gelatin–fibrin film formed was stored in a polythene cover. It is denoted as "GF."

Gelatin Graft Copolymer Impregnated with Fibrin (Crosslinked) Preparation

To a 10% gelatin solution, prepared as above, was added 0.25 g potassium persulfate, 0.25 g sodium metabisulfite, 2 mL butyl acrylate, and 4 mL HEMA. The mixture was stirred for 45 min, then 0.35 mL glutaraldeylde were added and the stirring was continued for 10 min. Later, 1 mL of ethylene glycol was added and stirred for another 5 min. Followed by this, 0.25 g fibrin powder was added and the stirring was ceased after 2 min. Finally, the content was poured into a polythene tray and dried at 40°C to form a film. The gelatin–polymer–fibrin composite film (crosslinked) was then washed with acetone to remove excess poly(hydroxyethyl methacrylate (pHEMA) and poly(butyl acrylate) (pBA) homopolymers and then dried and stored in polythene covers. This film is denoted as "GELF I."

Gelatin Graft Copolymer Impregnated with Fibrin (Uncrosslinked) Preparation

The procedure mentioned above (GELF I) was followed except for the addition of glutaraldehyde. The product obtained here is denoted as "GELF II."

Characterization

The analyses of the products prepared [gelatin (G), fibrin (F), GELF I, and GELF II) were carried out for studying the moisture content, equilibrium water absorption, percentage grafting, tensile strength, and infrared spectroscopy.

Moisture content

2–4 g of the material was taken in a china dish and dried at 100–105°C for 5 h. Then it was cooled

in a desiccator and weighed to a constant weight. Loss in weight was reported as moisture.

Water absorption capacity

Estimation of water absorption capacity was done by the method explained by Rao, Joseph, and Nayudamma.[17] The water absorption capacity of G, F, GELF I, and GELF II 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, and 3 h, 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.

Percentage grafting is determined by the following equation:

$$\% \text{ grafting} = \frac{[\text{Total weight of graft copolymer} - (\text{weight of the G + F})]}{\text{weight of the G + F}} \times 100$$

Tensile strength

Two dumbbell-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 testing system according to Vogel (1971)[18] at an extension rate of 10 cm/min.

Infrared spectroscopy

To provide proof of grafting the infrared spectra of gelatin, fibrin, and graft copolymer of G + F composite were measured with Nicolet impact 400 Fourier transform infrared spectroscopy (FTIR) using a 500-mg KBr pellet containing 2–6 mg of the sample.

Table I Moisture Contents of the Films

S. No.	Sample	% Moisture
1.	G	10.5
2.	F	11.6
3.	GF	12.58
4.	GELF I	11.52
5.	GELF II	9.37

RESULTS AND DISCUSSION

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. Chemically modified gelatin, which is impregnated with fibrin, offers the required characteristics that are essential for an ideal wound dressing. In the present study, the gelatin was graft-copolymerized and made in the form of film after fibrin was incorporated in it. The membranes thus prepared were characterized for their moisture content, equilibrium water absorption, percentage grafting, tensile strength, and infrared spectroscopy.

Moisture Content

The moisture contents of the films G, F, GF, GELF I, and GELF II are given in Table I. The moisture content varied between 9.37 to 12.58. This is the ideal moisture content for the biomaterial to store at room temperature. At this moisture level, the possibility of bacterial attack is less.

Water Absorption Capacity

Previously, chemically modified gelatin sheets were used on wounds.[19] Sometimes the sheets did not adhere to the wound properly and absorption was very poor. To circumvent this problem the gelatin was graft-copolymerized by butyl acrylate and poly(hydroxyethyl methacrylate), in this article. The equilibrium water absorption values of G, F, GF, GELF I, and GELF II are given in Table II.

The water absorption capacity values of crosslinked and uncrosslinked G + F graft copolymers were more or less the same. The film, made of gelatin alone and the gelatin film impregnated with fibrin were disintegrated within 1 h. Whereas fibrin film has shown 42–47% water ab-

sorption and it was stable in water even upto 24 h, GELF I and GELF II have shown an excellent water absorption capacity of 471% and 465%, respectively. This is due to the introduction of OH groups (provided by pHEMA) on the backbone.[20] This hydroxylated product (hydrogel) is stable and will have better wound exudate absorbing capacity.

Percentage Grafting

The grafting of G + F was carried out using potassium persulfate and sodium metabisulfite as initiators. The results of the present investigation indicate that the butyl acrylate and poly(hydroxyethyl methacrylate) were readily grafted onto gelatin. The percentage of grafting of GELF I was 32.19 and that of GELF II was 30.93, as seen from Table III. From these results it is clear that the percentage of grafting in the case of glutaraldehyde crosslinked product (GELF I) is slightly higher than the uncrosslinked sample.

Infrared Spectroscopy

The infrared spectra of gelatin [Fig. 1(a)], fibrin [Fig. 1(b)], HEMA-BA copolymer [Fig. 1(c)], and GELF I [Fig. 1(d)] were compared. The gelatin and fibrin, being proteins, showed the characteristic amide absorption bands at 1660 cm^{-1}, 1550 cm^{-1}, and 1250 cm^{-1} [Fig. 1(a, b)]. In another IR spectrum of HEMA-BA copolymer [Fig. 1(c)], the absorption band at 1720 cm^{-1} characteristic of ester carbonyl was seen. The spectrum taken for gelatin-grafted copolymer (GELF I) as in Figure 1(d) showed absorption bands responsible for both amide as well as ester carbonyl groups. It is understood from these results that the pHEMA and pBA were grafted onto the gelatin. As mentioned in Experimental the presence of free homopolymer, if any, in the GELF I was washed with acetone. The grafting of polymer onto the protein is also confirmed by the results of percentage of strain at break. Several investigators have reported that selected polymer-grafted proteins are biocompatible and find use in varieties of medical devices.[21–23]

Table II Water Absorption Capacity of Films

S. No.	Sample	% Water Absorption 1 h	2 h	3 h	24 h
1.	Fibrin (F)	42	44	47	47
2.	Gelatin (G)		film dissolved		
3.	G + F		film dissolved		
4.	GELF I	194	196	241	471
5.	GELF II	190	190	238	465

Table III Percentage Grafting

S. No.	Sample	% Grafting in the Graft Copolymer of G + F
1.	GELF I	32.19
2.	GELF II	30.92

Tensile Strength

Glutaraldehyde is widely used in protein chemistry as a crosslinking agent and it reacts primarily with amino groups of protein. It has been used in the manufacture of medical devices, tissue prostheses, and as a sterilizing agent.[24] Glutaraldehyde is also being used for improving the mechanical strength of collagenous and gelatinous materials.[20] With this view, the crosslinking agent was used here to obtain a biomaterial with enhanced mechanical properties and relatively increased resistance to biodegradation. The tensile strength data of the films of G, F, GF, GELF I, and GELF II are shown in Table IV.

The samples of G and F could not be prepared (punched out from film) for tensile strength measurement due to their brittleness. Even though the tensile strength of GF is as high as 20.97 N/mm^2, the percentage strain at break is very much less when compared to that of the grafted samples. Contrary to this, the grafted samples, GELF I and GELF II, displayed a low tensile strength (4.02 and 3.67 N/mm^2, respectively) and high percentage strain at break values. The percentage strain at break of GELF I is 19 times and GELF II is 11 times higher than that of GF. This observation shows that the grafted materials are more elastic and stable when subjected to mechanical force compared to the ungrafted GF. This property of grafted material is considered more suitable for a wound dressing substance that behaves like skin and adheres to the wound. However, the crosslinked film, GELF I, is considered the best of all other samples in this article as it showed a 40% increase in the percentage strain at break.

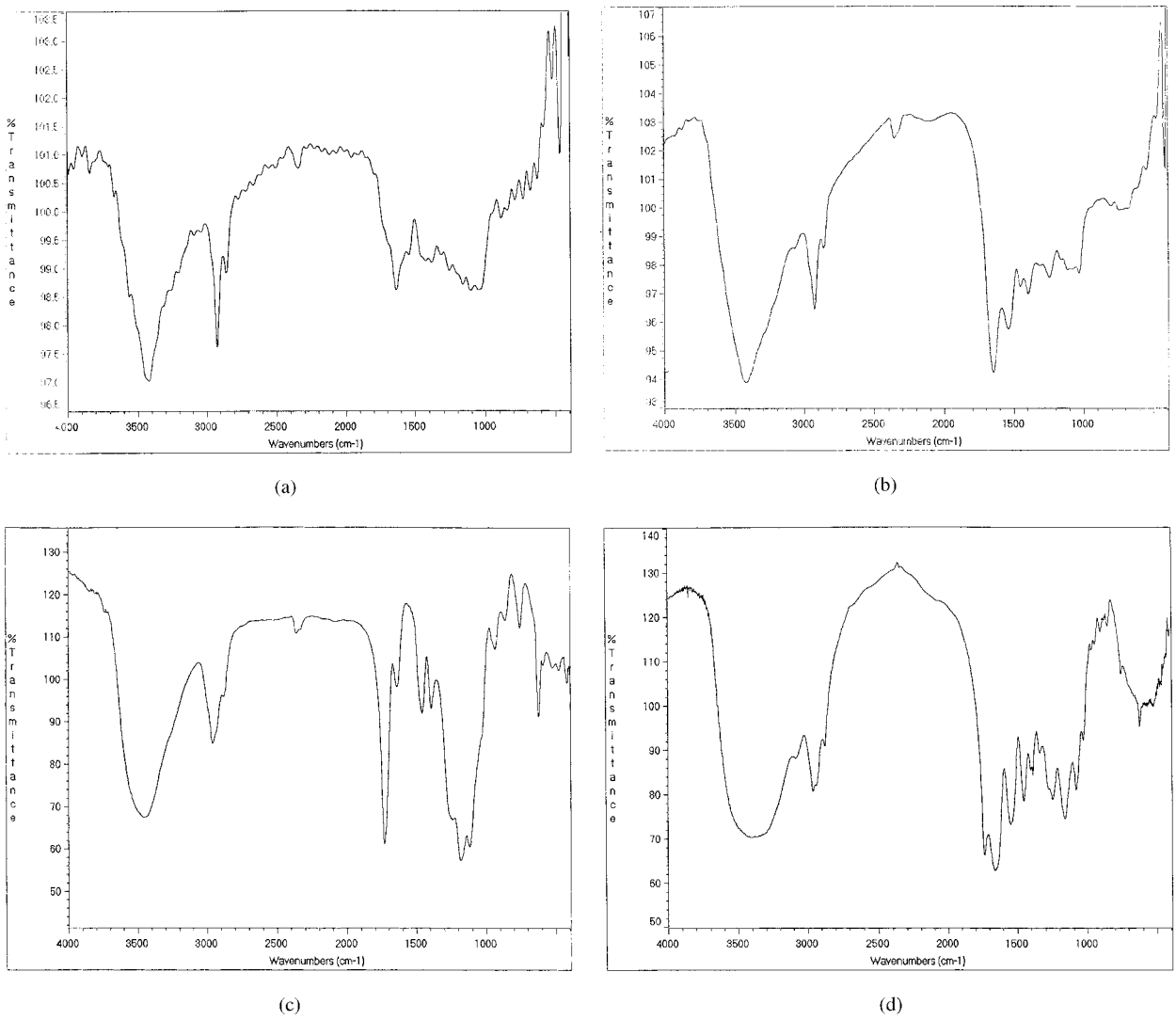

Figure 1 Infrared spectrum of (a) gelatin (G); (b) fibrin (F); (c) HEMA-BA copolymer; (d) Gelatin-HEMA-BA graft copolymer impregnated with fibrin (GELF I).

CONCLUSIONS

Grafting of polymers onto the backbone in the GELF I (gelatin graft copolymer impregnated with fibrin) preparation was evident from the IR spectrum. The increased water absorption properties of GELF preparations, particularly the GELF I, reflect increased water absorption capacity of the film, which could therefore serve as a better hemostat/wound dressing material.

Any biomaterial prepared should have sufficient strength for handling during surgical operations. At the same time it should not be easily biodegradable. The tensile strength obtained for GELF I indicated the workable mechanical properties of the film. The elastic nature (increased percent strain at break) of this hydrogel reflects the property of the natural skin, which is essential

Table IV Mechanical Properties of the Films

S. No.	Sample	Tensile Strength N/mm^2	% Strain at Break
1.	G	Brittle	
2.	F	Brittle	
3.	GF	20.97 ± 0.20	28.93 ± 2.82
4.	GELF I	4.02 ± 0.45	554.85 ± 4.05
5.	GELF II	3.67 ± 0.13	331.40 ± 0.42

for a wound dressing material in order to cover the contours of the wound and absorb the exudate.

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