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Biomaterials 26 (2005) 6335–6342
Biomaterials
www.elsevier.com/locate/biomaterials

Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin

Biji Balakrishnan[a], M. Mohanty[b], P.R. Umashankar[c], A. Jayakrishnan[a,*]

[a]*Division of Polymer Chemistry, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Satelmond Palace Campus, Trivandrum, Kerala 695 012, India*
[b]*Division of Implant Biology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Satelmond Palace Campus, Trivandrum, Kerala 695 012, India*
[c]*Division of Vivarium, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Satelmond Palace Campus, Trivandrum, Kerala 695 012, India*

Received 15 February 2005; accepted 4 April 2005
Available online 24 May 2005

Abstract

Wound dressings that can be formed in situ offer several advantages over the use of preformed dressings such as conformability without wrinkling or fluting in the wound bed, ease of application and improved patient compliance and comfort. Here we describe such an in situ forming hydrogel wound dressing from gelatin, oxidized alginate and borax. Periodate oxidized alginate rapidly cross-links proteins such as gelatin in the presence of borax to give in situ forming hydrogels that are both non-toxic and biodegradable. The composite matrix has the haemostatic effect of gelatin, the wound healing-promoting feature of alginate and the antiseptic property of borax to make it a potential wound dressing material. The hydrogel was found to have a fluid uptake of 90% of its weight which would prevent the wound bed from accumulation of exudates. The water vapour transmission rate (WVTR) of the hydrogel was found to be $2686 \pm 124\,g/m^2/day$ indicating that the hydrogel can maintain a moist environment over wound bed in moderate to heavily exuding wound which would enhance epithelial cell migration during the healing process. The wound healing efficacy of hydrogel was evaluated in experimental full thickness wounds using a rat model which demonstrated that within 2 weeks, the wound covered with gel was completely filled with new epithelium without any significant adverse reactions. These in situ forming hydrogels fulfil many critical elements desirable in a wound dressing material.

Keywords: Alginate; Gelatin; Cross-linking; Hydrogel; Wound dressing; Wound healing; In vivo test

1. Introduction

Wound healing is a dynamic process and the performance requirements of a dressing can change as healing progresses. However, it is widely accepted that a warm, moist environment encourages rapid healing and most modern wound care products are designed to provide these conditions [1,2]. Fluid balance in burn injury is very important since heavy loss of water from the body by exudation and evaporation may lead to a fall in body temperature and increase in the metabolic rate. Besides this, dressing should have certain other properties like ease of application and removal, and proper adherence so that there will not be any area of non-adherence left to create fluid-filled pockets for the proliferation of bacteria [3].

Numerous wound dressing materials are available and are also being investigated [3–8]. Hydrogels combine the features of moist wound healing with good fluid absorbance and are transparent to allow the monitoring of healing. In situ forming hydrogels that mould into the shape of wound defect will have advantages over the use

*Corresponding author. Tel.: +91 471 2340801; fax: +91 471 2341814.
E-mail address: dr_jkrishnan@sify.com (A. Jayakrishnan).

0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.04.012

of preformed hydrogel scaffolds since it would enable conformability of the dressing on wounds without wrinkling or fluting. Most commercially available dressings in the form of membranes and sheets are problematic as far as the conformability is concerned and the in situ formed dressings will therefore be superior to preformed dressings.

Spray-on films have been in existence since 1950s but were reported to produce problems of bacterial spread when used over open third degree wounds [4]. 'Hydron' is a commercial dressing based on poly(2-hydroxyethyl methacrylate) and polyethylene glycol that is formed in situ on the wound by spraying, but their high cost limits their use over simpler dressings [9,10]. Another dressing, based on block copolymers of ethylene and propylene oxides that forms a gel at body temperature has been tested as a burn dressing on rats [11,12]. A gelatin-based spray-on foam bandage has also been reported [13]. Adipic dihydrazide derivatives of chondroitin sulphate and hyaluronic acid that cross-link with poly(ethylene glycol) propiondialdehyde rapidly have been investigated by Kirker et al. [14].

In a recent study, we showed that the presence of small concentrations of borax accelerated the reaction between periodate-oxidized alginate and gelatin to give in situ forming hydrogels rapidly [15]. These gels were non-toxic and biodegradable and we demonstrated their potential as injectable in situ forming scaffolds for tissue engineering and drug delivery. Both alginate [16–19] as well as gelatin [20–23] have been used in a number of biomedical applications such as wound dressings, tissue engineering and drug delivery. 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. Production of these cytokines at the wound site results in a pro-inflammatory stimulus advantageous to wound healing. The high level of bioactivity of these dressings is believed to be due to the presence of endotoxin in alginates [24]. Gelatin sponges are used for inducing haemostasis in bleeding wounds. Therefore, a composite matrix derived from alginate and gelatin will have the synergic beneficial aspects of both polymers. Borax also has long history of medical use because of its antiseptic and antiviral activity and aqueous solutions have been used as mouth washes, eye-drops, skin lotions and cosmetics and in ointments. It has been reported that a number of metabolic processes are beneficially affected by physiological amount of dietary boron. Dietary boron helps in controlling the normal inflammatory process by serving as a signal suppressor that down-regulates specific enzymatic activities typically elevated during inflammation [25]. The present study was therefore undertaken in order to evaluate the oxidized alginate/gelatin system as an in situ forming wound dressing material. The material was found to possess many critical elements desirable in a wound dressing such as good water absorptivity, conformability, optimal water vapour transmission rate, mild antiseptic properties and biodegradability.

2. Materials and methods

2.1. Materials

Medium viscosity (viscosity of 2% solution 3500 cps at 25 °C) sodium alginate from the brown algae *Macrocystis pyrifera*, gelatin (Type A, Bloom 300, MW 100,000) and sodium tetra-borate decahydrate (Borax) were obtained from Sigma Chemical Co., St. Louis, MO, USA. Dynaplast, elastic adhesive bandage was obtained from Johnson & Johnson Ltd., Mumbai, India. Ketamine hydrochloride and pentabarbitol were procured from Neon laboratories Ltd., Mumbai, India. Xylaxin for injection was obtained from Indian Immunologicals Ltd., Hyderabad, India. Medical grade ethanol was locally obtained and was distilled before use. All other reagents used were of analytical or equivalent grade. Singly distilled water was employed throughout in all in vitro experiments. Phosphate-buffered saline (PBS, pH 7.4, 0.1 M) was prepared by dissolving 17.97 g of di-sodium hydrogen phosphate, 5.73 g of monosodium hydrogen phosphate and 9 g of sodium chloride in 1 L distilled water.

2.2. Methods

2.2.1. Preparation of alginate dialdehyde cross-linked gelatin hydrogels

Periodate oxidation of sodium alginate and purification and characterization of the resulting alginate dialdehyde (ADA) have been reported earlier in detail [15]. ADA was made to react with gelatin to form the cross-linked gel in the presence of 0.1 M borax. Gels were prepared by using a double syringe fibrin glue applicator, in which one syringe was filled with the solution of ADA in 0.1 M borax and the other with equal volume of gelatin in water. The applicator was fitted with a 20 G needle. The mixing of the polymer solutions inside the hypodermic needle on pushing the common plunger in the applicator led to gelation and cross-linking in a few seconds leading to the formation of the hydrogel.

2.2.2. Fluid uptake ability of hydrogels

The fluid absorbing capacity of the hydrogel is one of the important criteria for maintaining a moist environment over wound bed. One half millilitre of a 20% solution of ADA having a degree of oxidation 57% in 0.1 M borax and an equal volume of 15% solution of gelatin in water were introduced into screw-capped test

tubes of 10 mL capacity using the fibrin glue applicator. Gelation occurred within seconds after the mixture was extruded out of the needle. After 10 min, 5 mL PBS was introduced and the tubes were incubated at 37 °C. At regular intervals of time, the weight of the gel was noted after removing the PBS and gently blotting the gels with a filter paper. Weights of gels were noted until equilibrium swelling was reached.

$$\text{Equilibrium fluid content}\,(\%) = \frac{[W_s - W_d]}{W_s} \times 100,$$

where W_s and W_d represent the weight of swollen and dry sample, respectively. All experiments were done at least in triplicate.

2.2.3. Water vapour transmission rate

The moisture permeability of the hydrogel was determined by measuring the water vapour transmission rate (WVTR) across the material as stipulated by ASTM standard [26]. Gels having a diameter of 35 mm were prepared by mixing 2 mL of 57% oxidized alginate in 0.1 M borax and equal volume of 15% gelatin solution in tissue culture wells. The hydrogels were mounted on the mouth of cylindrical plastic cups (34 mm diameter) containing 10 mL water with negligible water vapour transmittance. The material was fastened using Teflon tape across the edges to prevent any water vapour loss through the boundary and kept at 37 °C and 35% relative humidity in an incubator. The assembly was weighed at regular intervals of time and weight loss versus time plot was constructed. From the slope of the plot, WVTR was calculated by the following formula,

$$\text{WVTR} = \frac{\text{slope} \times 24}{A}\ \text{g/m}^2\text{/day},$$

where A is the test area of the sample in m^2. Experiments were done in triplicate.

2.2.4. Rate of evaporation of water from gel

Hydrogels prepared as described above were kept at 37 °C and 35% relative humidity. After regular intervals of time, the weight was noted. Weight percentage was found out by the equation:

$$\text{Weight remaining}\,(\%) = \frac{W_t}{W_0} \times 100,$$

where W_0 and W_t are initial weight and weight after time 't' respectively.

2.2.5. In vivo wound healing

The wound healing characteristics of the in situ formed hydrogel were evaluated using a rat model. All experiments were performed with the approval of the Institute's Animal Ethics Committee. Male Wistar rats weighing approximately 250 g was anesthetized by intramuscular injection of Ketamine and Xylaxin, at a dose of 40 and 5 mg/kg body weight, respectively. The skin of the animal was shaved and disinfected using 70% ethanol. Two full thickness skin wounds of 1 cm^2 area were prepared by excising the dorsum of the animals. The wound was photographed by placing a sterile ruler along its side to measure the wound area.

ADA, borax and gelatin were sterilized with ethylene oxide using standard protocols. Sterile, pyrogen-free distilled water was used to prepare solutions of ADA and of gelatin. About 0.2 mL of 20% solution of ADA having degree of oxidation 57% in 0.1 M borax and an equal volume of 15% solution of gelatin were introduced onto the wound bed using the double syringe fibrin glue applicator. Spreading of the gel evenly on the wound bed was done immediately on application with the aid of fire-polished glass rod tip. The test wounds ($n = 6$) were then covered with sterile gauze, which was then fixed with elastic adhesive bandage (Dynaplast®). Similarly, control wounds ($n = 6$) also were covered with sterile gauze and elastic adhesive bandage without the test material. After experiment, animals were kept in separate cages and fed with commercial rat feed and water ad libitum until they were sacrificed.

The rats were sacrificed by excess dose of sodium pentabarbitol on day 5, 10 and 15 after surgery. The wounds were grossly examined and photographed for measurement of wound size reduction. For histology, the skin including the entire wound with adjacent normal skin was excised and fixed in 10% buffered formalin. The specimen included the dermis and the subcutaneous tissue. The wound size measurements taken at the time of surgery and at the time of biopsy were used to calculate the percent reduction in wound size using equation

$$\text{Wound size reduction}\,(\%) = \frac{[A_0 - A_t]}{A_0} \times 100,$$

where A_0 and A_t are initial wound area and wound area after a time interval 't'. Area was measured from the photographs of the wounds using the image analysis software (NIH Image tool III, Maryland, USA).

2.2.6. Histology

Excised wound sites fixed in formalin were processed and embedded in paraffin, and sections of 3–5 μm were stained with hematoxylin and eosin. Percentage of wound re-epithelialization was determined by using image analysis (Optimas™ 6.1, West Ford, MA, USA). The distance from right wound margin to left wound margin was measured. The length of newly generated epithelium across the surface of the wound was determined as the sum of the new epidermis growing from right and left margins of the wound. This length was expressed as a percentage of entire wound length.

2.2.7. Statistical analysis

Statistical analysis of data was performed by one way analysis of variance (ANOVA), assuming confidence level of 95% ($p<0.05$) for statistical significance. All the data were expressed as mean ± standard deviation (SD).

3. Results and discussion

The periodate oxidation of sodium alginate, its characterization and the kinetics of gelation of ADA with gelatin have been extensively reported earlier [15]. A 20% solution of ADA having degree of oxidation 57% and a 15% solution of gelatin were optimal with respect to dissolution, ease of handling and gelation time in the preparation of hydrogels for many applications. Therefore, all experiments were done with 57% oxidized alginate. ADA was cross-linked with gelatin in the presence of 0.1 M borax to form hydrogels without the use of any extraneous cross-linking agents. Cross-linking is predominantly due to Schiff's base formation between the ε-amino groups of lysine or hydroxylysine side groups of gelatin and the available aldehyde. The presence of borax facilitates the Schiff's base formation due to the alkaline pH (9.4) and enhanced solubility of ADAs due to complexation [15].

The fluid uptake ability of ADA cross-linked gelatin hydrogel was evaluated by incubating in PBS at 37 °C. Fig. 1 shows the kinetics of swelling of hydrogel. Initial fluid content of the gel is about 85%. This is in agreement with the concentrations of the reacting solutions employed. Noteworthy here is the fact that on cross-linking, the gel does not exude the fluids and retain the initial amount present. On equilibration, the swelling increased to only about 90%. This was interesting from the point of gel strength; a significant swelling following equilibration would lead to poor mechanical properties.

An ideal wound dressing must control the water loss from a wound at an optimal rate. Lamke et al. [27] reported the evaporative water loss for normal skin as $204 \pm 12\,g/m^2$/day and that for injured skin can range from $279 \pm 26\,g/m^2$/day for a first degree burn to $5138 \pm 202\,g/m^2$/day for a granulating wound. The water vapour permeability of a wound dressing should prevent excessive dehydration as well as build up of exudates. It has been recommended that a rate of 2000–2500 g/m^2/day would provide adequate level of moisture without risking wound dehydration [28]. WVTR of the hydrogel was calculated as the gradient of the weight loss versus time plot. Fig. 2 shows the loss of water vapour with time through the hydrogel when placed in a moisture-rich environment. Wound dressings available in market such as Geliperm® (Geistlich Ltd., Switzerland) and Vigilon® (Bard Ltd., Crawley, UK) were found to have a WVTR of 9009 ± 319 and $9360 \pm 34\,g/m^2$/day, respectively, and thus acts as water-free surface [29]. Such high WVTR would lead to total dehydration of the wound surface enabling the dressing adhere to the wound. The hydrogel in the present investigation showed a value of $2686 \pm 124\,g/m^2$/day close to the range appropriate to maintain a proper fluid balance on the wound bed, which can facilitate cellular migration and enhance re-epithelialization.

The extent of water loss from the hydrogel on exposure to the air was evaluated to examine its

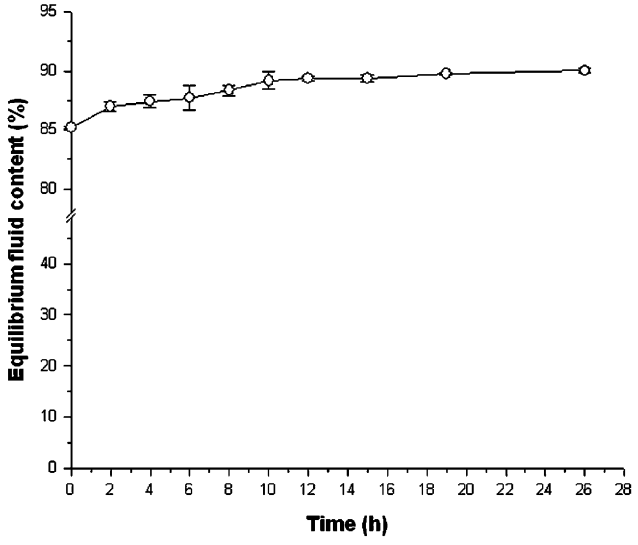

Fig. 1. Equilibrium PBS content of hydrogel prepared by cross-linking 15% solution of gelatin with 20% solution of ADA having degree of oxidation of 57% in 0.1 M borax.

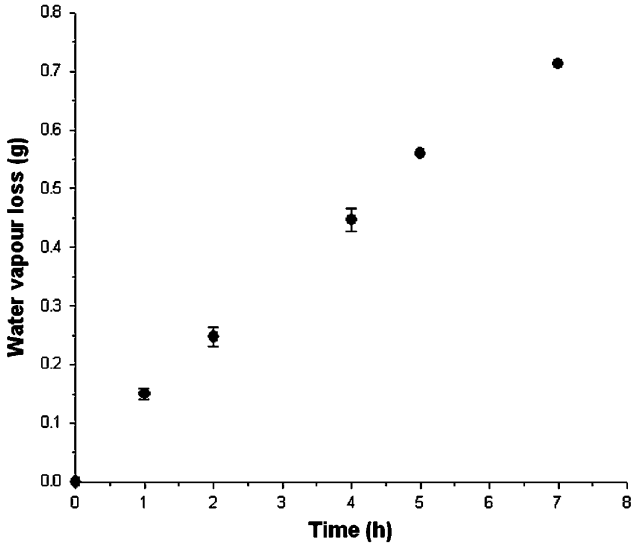

Fig. 2. Water vapour transmission loss from hydrogel prepared by cross-linking 15% solution of gelatin using ADA having degree of oxidation 57% in 0.1 M borax.

behaviour when used as a dressing over a dry wound. It was found that the loss of water increased linearly with time for the first 2 days. After 1 day, the loss was approximately 30–40% and this increased slowly to about 80% over 4 days (Fig. 3). Subsequently, there was no water loss from the gel, and the gel retained about 10–15% of water. From the gradient of water loss versus time plot, the amount of water loss from the gel in $g/m^2/h$ was estimated. The hydrogel showed different rates of evaporation at different intervals of time. It was found that up to 5 h, the gel lost water at a rate of $20\,g/m^2/h$. On the next 2 days, a rate of $15\,g/m^2/h$ was observed. After that, the rate decreased to $7\,g/m^2/h$. It is clear from these studies that the material will lose its water content when exposed to air under dry conditions over long periods. Thus, these dressings will be more beneficial to wounds with moderate exudates rather than for dry wounds. It may be pointed out that the dressings can be kept moist if desired by spraying saline or water since these hydrogels rapidly imbibe water. Commercially available dressing like Geliperm® (Geistlich Ltd., Switzerland) has also been reported to show similar behaviour losing about 50% of its bound water after 12 h, and retaining about 30% water after 24 h. It has been reported that this water loss enables the gel to take up exudates and oedema fluid from the wound into the dressing by an active upward-directed process when used in exudating wounds [8].

The wound healing efficacy of the hydrogel was evaluated in a full thickness rat wound model.

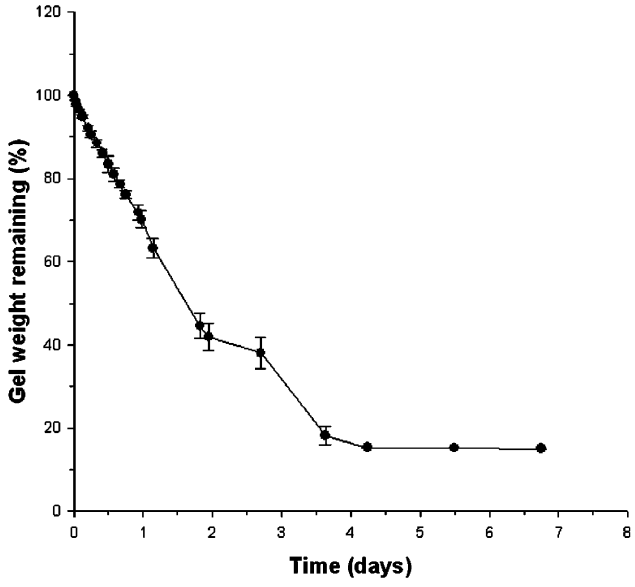

Fig. 3. Evaporative water loss from hydrogels prepared by cross-linking 15% solution of gelatin using ADA having degree of oxidation 57% in 0.1 M borax.

3.1. Gross examination

Grossly, each wound (both test and control) was observed for a period of 5, 10 and 15 days post treatment (Fig. 4). At 5 days, subcutaneous aspect appeared grossly normal for the test samples and there was no evidence of infection or contraction of the wound, while skin was haemorrhagic for some control samples and also scab was present on the wound bed. It has been reported that epithelialization is retarded by

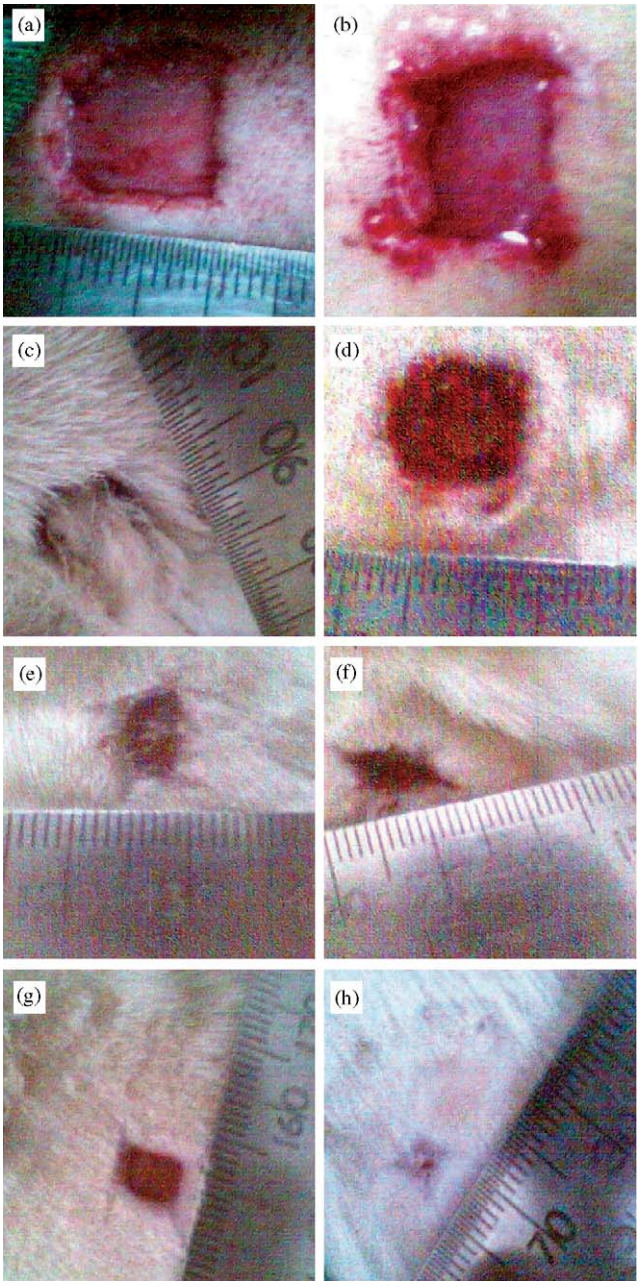

Fig. 4. Representative photographs of macroscopic appearance of 1×1 cm wound excised on rat (a), gel applied on wound (b), control wounds at 5 (c), 10 (e) and 15 days (g) and test wounds at 5 (d), 10 (f) and 15 days (h).

Table 1
Percent wound size reduction and rate of wound re-epithelialization at 5, 10 and 15 days of post wounding

Days of observation	Wound size reduction ± SD (%)		Wound re-epithelialization ± SD (%)	
	Test	Control	Test	Control
5	—	—	42.16 ± 9	11 ± 6
10	68.9 ± 2	72.5 ± 6	85.23 ± 11	74.6 ± 27
15	95.3 ± 5	75.5 ± 5	90.38 ± 9	81.65 ± 9

the dry scab. Winter showed that epithelialization can be accelerated if the wound is kept moist [1]. One explanation for this was that keratinocytes migrated more easily over a moist wound surface than underneath a scab [30]. Epidermal cells can migrate at a speed of about 0.5 mm/day over a moist wound surface which is twice as fast as under a scab in dry wounds [31]. Subcutaneous aspects appeared grossly normal for test and control wounds at 10 days of post wounding. At 15 days, majority of the test wounds appeared to be healed.

By measuring the wound area before and after definite intervals of time, reduction in wound defect area was calculated. At 5 days, there was no reduction in wound defect area for both test and control. At 10 days, healing started leading to about 72% fill in wound defect for control wounds, whereas for test wounds this was only 68.9% (Table 1). Statistical analysis revealed that this difference was not significant ($p > 0.05$). However, at 15 days, wound defect filled up to 95.3% in the case of test wounds, whereas for control wounds this was about 75% which was statistically significant ($p < 0.05$).

3.2. Histological examination

Healing pattern of wounds was studied by examining the histology of the test and control samples at 5, 10 and 15 days of post wounding.

3.3. Fifth day of observation

In the superficial layers, moderate necrosis with severe inflammation was observed in both test and control wounds during this period (Fig. 5a and b). Inflammatory granulation tissue was seen in dermis. Inflammatory phase is a normal and necessary prerequisite to healing [32]. This can be initiated by numerous causes, one of which is injury. Therefore, during early stage of wound healing, it is difficult to assess whether the inflammatory response is part of normal healing process or due to the effect of material. During this stage macrophages are the notable feature at the wound site. In test wounds during this period, foreign body type giant cell response was observed in the dermis. It is reported that as a consequence of macrophage/biomaterial interactions, there is fusion of adherent macrophages leading to the formation of multinucleated foreign body giant cells (FBGC) on biomaterial surfaces [33,34]. This phenomenon is accompanied by FBGC-mediated biomaterial degradation [35,36] which is believed to result from the action of reactive oxygen species within an acidified closed compartment between FBGC and the biomaterial substrate [37].

Focal irregular areas of new epithelium on edge were also noted for some of the test wounds during this period (Fig. 5a), whereas it was found only in one of the control wounds. In test samples collagen appeared mature in lower dermis. Bacterial colonies were found in control wounds (Fig. 5b), whereas no bacterial colony was found in any of the test wounds. The absence of bacterial colonies leading to infection in test wounds is noteworthy and is attributed to the mild antiseptic properties of borax present in the matrix. The presence of borax also therefore serves the important function of preventing bacterial infection of the wound bed.

3.4. Tenth day of observation

At 10 days, test wounds appeared reduced in size with new epithelium noted at both the edges of the defect with the proliferation of basal layer and formation of the rete pegs (Fig. 5c and d). New collagen formed in the dermis appeared mature. Granulation tissue was seen in dermis. Granulation tissue formation is essential for permanent wound closure, since it fills the defects and prepares the way for epithelialization. These findings support that ADA/gelatin hydrogel is able to provide suitable condition for granulation tissue formation.

3.5. Fifteenth day of observation

At 15 days, in test wounds, the defect area became small and filled with fibro-proliferative tissue. Inflammatory cells were absent. The entire surface of the defect was covered with new epithelium. Mature collagen was present in dermis. Fig. 5e shows the presence of mature collagen under polarised light. However, for some control wounds though the entire surface of the defect was covered with new epithelium, moderate number of inflammatory cells, predominantly lymphocytes and macrophages, were still present in the upper dermis. Immature collagen fibres filled the dermis (Fig. 5f).

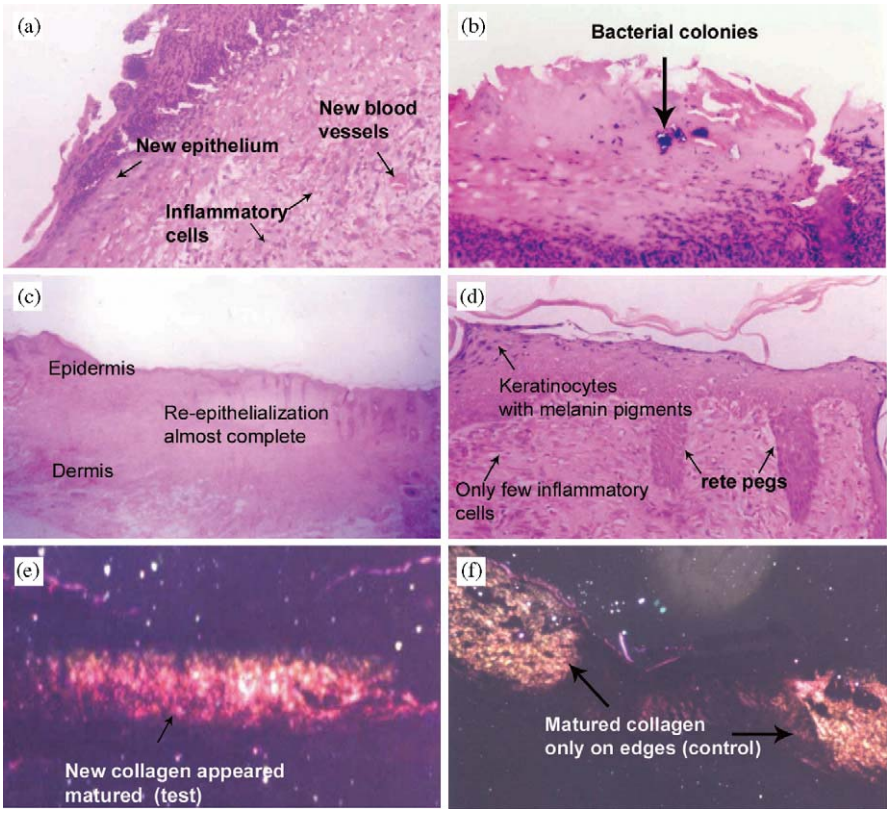

Fig. 5. Histology of wound sections stained with hematoxylin and eosin. Epithelialization at test wound edges at 5 days (a, 150 ×); bacterial colonies present in control wounds (b, 300 ×); neat test wound section at 10 days (c, 60 ×); test wound with rete pegs at 10 days (d, 150 ×); test wound (e, 15 ×) and control wound (f, 15 ×) under polarised light at 15 days.

There are reports of FBGC reactions 7 months after the use of calcium sodium alginate dressing (Kaltostat) [38]. However, in the present study, foreign body reaction subsided after 15 days demonstrating that the material was undergoing degradation on the wound bed and the degradation products were not inducing any adverse reaction within the body. We have shown earlier [15] that the ADA-cross-linked gelatin network is completely degradable under physiological environment unlike calcium cross-linked alginates which are less susceptible to biodegradation and have a long residence time in the body.

3.6. Wound re-epithelialization

The length of newly generated epithelium across the surface of the wound was determined as the sum of the new epidermis growing from right and left margins of the wound and was expressed as a percentage of entire wound length. Though superficially neither control nor test wounds showed any reduction in defect area at 5 days, on measuring the wound re-epithelialization it was found that both wounds have started healing. However, due to scab formation over control wounds, the rate of epithelialization was slightly lower in control wounds than in test wounds (Table 1). At 10 days, the rate of re-epithelialization increased to $85.23 \pm 11\%$ for test wounds; whereas for control wounds, this was $74.6 \pm 27\%$. The rate of re-epithelialization further increased to $90.38 \pm 9\%$ and $81.65 \pm 9\%$, respectively, for test and control wounds at 15 days. Statistical analysis however revealed that though there was significant difference between control and test wounds in rate of re-epithelialization at 5 days, the difference was not significant at 10 and 15 days due to the large standard deviation observed.

4. Conclusion

The in situ forming wound dressing reported in this investigation employs a very simple method to prepare hydrogels that combines the beneficial properties of both alginate and gelatin and eliminates the use of extraneous cross-linking agents. The presence of borax is believed to exert an antiseptic effect to prevent bacterial colonization of the wound. The WVTR and water absorptivity of the hydrogel were found to be highly optimal for maintaining a moist environment conducive for wound healing. The wound healing efficacy of these in situ forming hydrogels can be further improved by incorporating drugs or growth factors.

Acknowledgements

The authors thank the Director, SCTIMST, for permission to publish this manuscript. A Research Fellowship from the University Grants Commission, New Delhi, to B. Balakrishnan is gratefully acknowledged.

References

[1] Winter GD. Formation of the scab and the rate of epithelialization of superficial wounds in the skin of the young domestic pig. Nature 1962;193:293–4.
[2] Barnett SE, Irving SJ. Studies of wound healing and the effect of dressings. In: Szycher M, editor. High performance biomaterials. Lancaster: Technonic; 1991. p. 583–620.
[3] Quinn KJ, Courtney JM, Evans JH, Gaylor JDS, Reid WH. Principles of burn dressings. Biomaterials 1985;6:369–77.
[4] Kane JB, Tomkins RG, Yarmush ML, Burke JF. Burn dressings. In: Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, editors. An introduction to materials in medicine. San Diego: Academic; 1996. p. 360–70.
[5] Choi YS, Hong SR, Lee YM, Song KW, Park MH, Nam YS. Studies on gelatin-containing artificial skin: II. Preparation and characterization of crosslinked gelatin-hyaluronate sponge. J Biomed Mater Res (Appl Biomater) 1999;48:631–9.
[6] Wang PY, Samji NA. Characterisation of a dextran hydrogel wound dressing. Org Coat Plast Chem 1980;42:628–33.
[7] Azad AK, Sermsintham N, Chandrkrachang S, Stevens WF. Chitosan membrane as a wound-healing dressing: characterization and clinical application. J Biomed Mater Res Part B 2004;69B:216–22.
[8] Kichöfen B, Wokalek H, Scheel D, Ruh H. Chemical and physical properties of a hydrogel wound dressing. Biomaterials 1986;7:67–72.
[9] Husain MT, Akhtar M, Akthar N. Report on evaluation of hydron as burn wound dressing. Burns 1983;9:330–4.
[10] Nathan P, MacMillan BG, Holder IA. In situ production of a synthetic barrier dressing for burn wounds in rats. Infect Immun 1975;12:257–60.
[11] Schmolka IR. Artificial skin. 1. Preparation and properties of Pluronic F-127 gels for the treatment of burns. J Biomed Mater Res 1972;6:571–82.
[12] Nalbandian RM, Henry RL, Balko KW, Adams DV, Neuman NR. Pluronic F-127 gel preparation as an artificial skin in the treatment of third-degree burns in pigs. J Biomed Mater Res 1987;21:1135–48.
[13] Neumann PM, Zur B, Ehrenreich Y. Gelatin-based sprayable foam as a skin substitute. J Biomed Mater Res 1981;15:9–18.
[14] Kirker KR, Luo Y, Nielson JH, Shelby J, Prestwich GD. Glycosaminoglycan hydrogel films as biointeractive dressings for wound healing. Biomaterials 2002;23:3661–71.
[15] Balakrishnan B, Jayakrishnan A. Self cross-linking biopolymers as injectable in situ forming biodegradable scaffolds. Biomaterials 2005;26:3940–51.
[16] Lee KY, Bouhadir KH, Mooney DJ. Degradation behaviour of covalently cross-linked poly (aldehyde guluronate) hydrogels. Macromolecules 2000;33:97–101.
[17] Bouhadir KH, Alsberg E, Mooney DJ. Hydrogels for combination delivery of antineoplastic agents. Biomaterials 2001;22:2625–33.
[18] Bouhadir KH, Lee KY, Alsberg E, Damm KL, Anderson KW, Mooney DJ. Degradation of partially oxidised alginate and its potential application for tissue engineering. Biotechnol Prog 2001;17:945–50.
[19] Thomas S. Alginate dressings in surgery and wound management. J Wound Care 2000;9:56–60.
[20] Kuijpers AJ, Engbers GHM, Krijgsveld J, Zaat SA, Dankert J, Feijen J. Crosslinking and characterisation of gelatin matrices for biomedical applications. J Biomater Sci Polym Ed 2000;11:225–43.
[21] Tomihata K, Burczak K, Shiraki K, Ikada Y. Crosslinking and biodegradation of native and denatured collagen. In: Shalaby SE, Ikada Y, Langer RS, Williams J, editors. Polymers of biological and biomedical importance. Washington, DC: American Chemical Society; 1994. p. 540.
[22] Draye J-P, Delaey B, Van de Voorde A, Van Den Bulke A, Bogdanov B, Schacht E. In vitro release characteristics of bioactive molecules from dextran dialdehyde crosslinked gelatin hydrogel films. Biomaterials 1998;19:99–107.
[23] Kuijpers AJ, van Wachum PB, van Luyn MJA, Engbers GHM, Krijsveld J, Zaat SAJ, Dankert J, Feijen J. In vivo and in vitro release of lysozyme from cross-linked gelatin hydrogels: a model system for the delivery of antibacterial proteins from prosthetic heart valves. J Controlled Release 2000;67:323–36.
[24] Thomas A, Harding KG, Moore K. Alginates from wound dressings activate human macrophages to secrete tumour necrosis factor-α. Biomaterials 2000;21:1797–802.
[25] Hunt CD, Idso JP. Dietary boron as a physiological regulator of the normal inflammatory response: a review and current research progress. J Trace Elem Exp Med 1999;12:221–33.
[26] ASTM Standard E96-00. Standard test methods for water vapour transmission of materials. Annual book of ASTM standards, vol. 4.06. Philadelphia: ASTM; 2000.
[27] Lamke LO, Nilsson GE, Reithner HL. The evaporative water loss from burns and the water permeability of grafts and artificial membranes used in the treatment of burns. Burns 1977;3:159–65.
[28] Queen D, Gaylor JDS, Evans JH, Courtney JM, Reid WH. The preclinical evaluation of the water vapour transmission rate through burn wound dressings. Biomaterials 1987;8:367–71.
[29] Wu P, Fisher AC, Foo PP, Queen D, Gaylor JD. In vitro assessment of water vapour transmission of synthetic wound dressings. Biomaterials 1995;16:171–5.
[30] Winter GD, Scales JT. The effect of air drying and dressings on the surface of a wound. Nature 1963;197:91.
[31] Winter GD. In: Maibach HI, Rovee DT, editors. Epidermal wound healing. Chicago: Year Book Medical Publishers; 1972. p. 71.
[32] Kirsner RS, Eaglstein WH. The wound healing process. Dermatol Clin 1993;11(4):629–40.
[33] Murch AR, Grounds MD, Marshall CA, Papadimitriou JM. Direct evidence that inflammatory multinucleate cells form by fusion. J Pathol 1982;137:177–80.
[34] Anderson JM. Inflammatory response to implants. Am Soc Artif Intern Organs 1988;11:101–7.
[35] Zhao Q, Topham NS, Anderson JM, Hiltner A, Lodoen G, Payet R. Foreign body giant cells and polyurethane biostability: in vivo correlation of cell adhesion and surface cracking. J Biomed Mater Res 1991;25:177–83.
[36] Wiggins MJ, Wilkoff B, Anderson JM, Hiltner A. Biodegradation of polyether polyurethane inner insulation in bipolar pacemaker leads. J Biomed Mater Res 2001;58:302–7.
[37] Heiple JM, Wright SD, Allen NS, Silverstein SC. Macrophages form circular zones of close apposition to IgG-coated surfaces. Cell Motil Cytoskel 1990;15:260–70.
[38] Mathew IR, Brownie RM, Frame JW, Millar BM. Subperiosteal behaviour of alginate and cellulose wound dressing materials. Biomaterials 1995;16:265–74.