Chitosan Film Containing Fucoidan as a Wound Dressing for Dermal Burn Healing: Preparation and In Vitro/In Vivo Evaluation

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ABSTRACT

The aim of this study was to develop chitosan film containing fucoidan and to investigate its suitability for the treatment of dermal burns on rabbits. Porous films, thickness between 29.7 and 269.0 μm, were obtained by the solvent dropping method. Water vapor permeability (3.3-16.6/0.1 g), the swelling (0.67-1.77 g/g), tensile strength (7.1-45.8 N), and bioadhesion (0.076-1.771 mJ/cm^2) of the films were determined. The thinnest films were obtained with the lowest chitosan concentration ($P < .05$). The water absorption capacity of the films sharply increased with the freeze-drying technique. The film having the thickness of 29.7 μm showed the highest amount of moisture permeability (16.6 g/0.1 g). Higher chitosan concentration significantly increased tensile strength of the films ($P < .05$). Using higher concentration of lactic acid made films more elastic and applicable, and these films were selected for in vivo studies. Seven adult male New Zealand white rabbits were used for the evaluation of the films on superficial dermal burns. Biopsy samples were taken at 7, 14, and 21 days after wounding, and each wound site was examined macroscopically and histopathologically. After 7 days treatment, fibroplasia and scar were observed on wounds treated with fucoidan-chitosan film. The best regenerated dermal papillary formation, best re-epithelization, and the fastest closure of wounds were found in the fucoidan-chitosan film treatment group after 14 days compared with other treatment and control groups. It can be concluded that fucoidan-chitosan films might be a potential treatment system for dermal burns and that changing formulation variables can modulate the characterizations of the films.

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KEYWORDS: Fucoidan, chitosan, biopolymer, film, wound healing.

INTRODUCTION

Wound repairing is a complex process involving an integrate response by many different cell types and growth factors to achieve rapid restoration of skin integrity and protective function after injury.[1] Dermal substitution and wound healing are research areas of medicine in which there have been many recent advances, but neither the commercially available products nor the materials currently described in experimental studies are able to fully substitute for natural living skin.[2] On the other hand, healing of dermal wounds with macromolecular agents such as natural polymers is preferred to skin substitutes owing to many advantages such as biocompatibility, nonirritant and nontoxic properties, and ease and safety of the application on dermis.[3]

Chitosan has been used as a wound dressing in burn healing for proliferation and activation of inflammatory cells in granulation tissue and consequently for accelerating wound cleaning and re-epithelization properties.[4,5] On the other hand, fucoidan, sulphated polysaccharide commonly obtained from seaweeds, shows significant gel contraction-promoting, integrin expression-enhancing, and heparin activities.[6] Although a great number of studies on the different pharmacological properties of fucoidan are available, there is little information on the fucoidan-based system used in wound healing and it is limited to cell culture only.[7,8] The aims of this study were to prepare chitosan films containing fucoidan and to investigate the suitability of the films on dermal burns on rabbits.

MATERIALS AND METHODS

Materials

Fucoidan (molecular weight [MW] 80 kd from *Fucus vesiculosus*), chitosan (MW 750 kd, deacetylation degree ≥85%), lactic acid (85% wt/vol), and calcium chloride (anhydrous, minimum 96%) were purchased from Sigma Chemical Co (St Louis, MO); and propylene glycol, from Merck (Darmstadt,

Germany). All other reagents and solvents were of pharmaceutical grade. The materials were used as received.

Preparation of Fucoidan-Chitosan Films

Fucoidan-chitosan films were prepared according to authors' earlier report.[9] Chitosan was dissolved in lactic acid solution containing fucoidan and stirred overnight, and then propylene glycol (2.5%) was added as a plasticizer. The resulting solution was sonicated to remove air bubbles, dropped into a Petri dish (3.0 mL) after adjusting the temperature to 30ºC, and dried. After drying, the films were peeled off and stored in an airtight container at room temperature until further investigation. Several variables were investigated for the optimization of the film properties (Table 1). The thickness of the films at 5 different locations (center 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.

Scanning Electron Microscopy

The prepared films were mounted on metal grids with double-sided adhesive tape, coated with gold to ~500×10^{-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 25ºC ± 1ºC. The surface morphology of coated samples was examined by scanning electron microscopy (SEM; JEOL JSM-5200, Tokyo, Japan) at 20 kV.

Water Vapor Permeability of the Films

The borosilicate glass bottles (capacity, 30 mL; diameter of top hole, 22 mm; Paşabahçe, Istanbul, Turkey) were filled with anhydrous calcium chloride; then the films were tied onto the mouth of the bottles. All bottles were put in a desiccator containing saturated sodium chloride solution (65% ± 5% relative humidity [RH]) and stored in an oven (BE 400, Memmert, Schwabach, Germany) maintained at 25ºC ± 1ºC. The average area available for vapor permeation was 3.8 cm^2. After 21 days, the containers were weighed with a weighing balance (Scientech ZSA80; tare range, 0-80 g; sensitivity, 0.0001 g; Sartorius, Goettingen, Germany) 3 times.[10]

Table 1. Codes and Formulation Parameters of Fucoidan-Chitosan Films*

Codes	Chitosan Conc (%)	Fucoidan Conc (%)	Lactic Acid Conc (%)	Drying Conditions
A1	1.0	0.5	1.0	Oven (40°C)
A2	1.5	0.5	1.0	Oven (40°C)
A3	2.0	0.5	1.0	Oven (40°C)
A4	2.0	—	1.0	Oven (40°C)
B1	2.0	0.25	1.0	Oven (40°C)
B2	2.0	0.75	1.0	Oven (40°C)
C1	2.0	0.5	2.0	Oven (40°C)
D1	2.0	0.5	1.0	Room temperature (25°C)
D2	2.0	0.5	1.0	Freeze-dried

*Conc indicates concentration.

Water Absorption Capacity of the Films

The films were suspended in glass bottles containing 50 mL of phosphate-buffered saline (PBS) (pH 7.4) at room temperature. At an appropriate time interval, the films were taken out, and the excess water was removed carefully with filter paper then weighed immediately. Measurements were performed 3 times.[11]

Mechanical Properties

The mechanical properties of the films were measured using a texture analyzer (TA.XT*Plus*, Stable Micro Systems, Haslemere, Surry, UK) equipped with a 5-kg load cell by the method of Kalapathy et al.[12] A film strip (dimensions 2 × 1 cm) was held between 2 clamps and pulled by the top clamp at a rate of 0.5 mm/s. 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 Equations 1 and 2.

$$\text{Tensile Strength } (\text{N/mm}^2) = \text{Breaking Force (N)} / \text{Cross-sectional Area of Sample } (\text{mm}^2) \quad (1)$$

$$\text{Elongation at Break } (\%) = [\text{Increase in Length at Breaking Point (mm)} / \text{Initial Length(mm)}] \times 100 \quad (2)$$

In Vitro Bioadhesion Studies

The bioadhesive strength of the films was evaluated by the modified method of Wong et al.[13] The measurement was conducted with a texture analyzer equipped with a 5-kg load cell and bioadhesion test rig. Chicken back skin was used as a model tissue. As follows, the skin taken from a freshly slaughtered animal was used after the removal of all the fats and debris. The dermal tissue was fitted on the bioadhesion test rig, and then 100 μL of distilled water was applied on the surface of the tissue before the study. The tests were done at 37°C. The film was cut into a circular shape and attached to the P/10 cylindrical Perspex (Lucite International Ltd., Queens Gate, UK) probe with double-sided adhesive tape. The probe was lowered onto the surface of the tissue with a

constant speed of 1 mm/s^{-1} and contact force of 1 N applied. After keeping in contact for 30 seconds, the probe was then moved vertically upwards at a constant speed of 1 mm/s^{-1}. Work of adhesion (mJ/cm^2) and peak detachment force (N/cm^2) were calculated from force-distance plot using the Texture Exponent 2.0.6.0 software package of the TA.XT*Plus*. Each experiment was performed 3 times.

Skin Burn Wounds

The animal study was approved by the Institutional Committee of Selçuk University for Animal Care in Laboratory Research, and the animals were kept under standard laboratory conditions. Seven male New Zealand white rabbits (mean weight, 4.2 ± 0.3 kg) were used for the evaluation of superficial burn wounds. The backs of the rabbits were depilated and ketamine (25 mg/kg) and xylazine (1 mg/kg) were injected intramuscularly into the rabbits before a heated aluminum stamp was applied. The electrically heated stamp was maintained at a temperature of 80°C and applied for 14 seconds for forming a superficial burn wound (burn area, 3.8 cm^2).[14] Each rabbit (A-D) had 4 wounds: A was treated with chitosan film containing fucoidan (CFF, C1 formulation); B was treated with fucoidan solution (FS); C was treated with chitosan film without fucoidan (CF, A4 formulation); and D as a control group (CTRL) was not treated. The biopsy samples were taken at 7-, 14-, and 21-day intervals from each rabbit at the beginning of the study, and the degree of healing was evaluated macroscopically and histopathologically.

Histopathological Examination

The biopsies of skin samples (0.5 × 1.5 cm^2) were fixed in a 10% buffered formaldehyde solution then embedded in paraffin block and sectioned in 4-μm increments. The sections were made perpendicular to the anterior-posterior axis and perpendicular to the surface of the wound. The sections were positioned on a slide and stained with hematoxylin-eosin and Masson's trichrome reagents.

AgNOR Staining and Quantification Study

The nucleolar organizer regions (NORs) stained by silver are called argyrophilic NOR-associated proteins (AgNORs). The samples were prepared according to an earlier study.[15,16] The 4-μm section samples were cut from the paraffin block, dewaxed with xylene, and hydrated. The staining solution (≈0.3 mL, including $AgNO_3$) was immediately poured on each slide. After staining (14-20 minutes), the solution was poured off and the slides were washed with bidistilled water, placed for 10 minutes in a 5% (wt/vol) thiosulfate solution, rinsed, and dried. The AgNOR proteins appeared as well-defined black dots that were counted in 50 cells with each sample. Measurements were performed 3 times.

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 $P < .05$.

Results and Discussions

Characterization of the Films

The surface of fucoidan-chitosan films has a porous structure as shown in Figure 1. As shown in Table 2, the thicknesses of films were in the range of 29.7 μm to 64.0 μm, with the exception of freeze-dried films (269.0 μm). The film thickness was affected by all parameters ($P < .05$). The thinnest films were the membranes prepared with 1.0% chitosan concentration (29.7 μm, A1). The thickest film was obtained with the freeze-dried technique. In addition, film thickness was highly increased by enhancing the chitosan concentration in the formulation (A1-A3; $P < .05$; Table 2).

Water vapor permeability values of films changed between 3.3 and 16.6 g/0.1 g, as shown in Table 2. Water vapor permeability of the films was significantly decreased with increased in chitosan concentration in the formulation, and drying method was found to be an effective parameter for permeability ($P < .05$). Thickness and water vapor permeability of the films vary in inverse proportion ($P < .05$). The

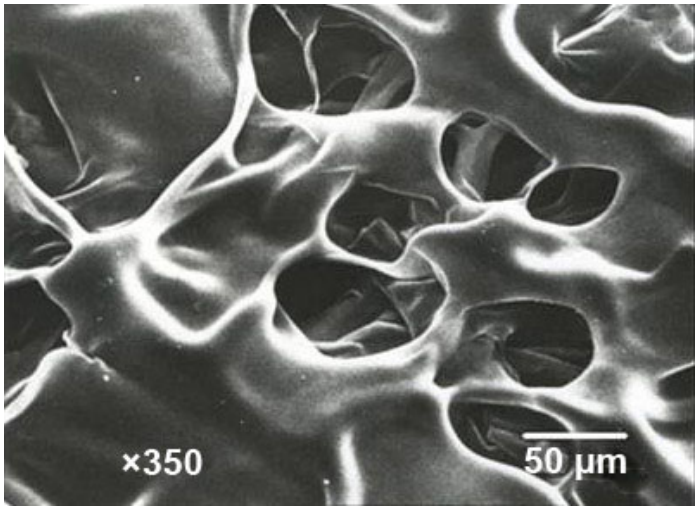

Figure 1. Scanning electron photomicrographs of fucoidan-chitosan film surface morphology (C1 formulation, chitosan film containing fucoidan).

Table 2. Physical, Mechanical, and Bioadhesive Properties of Fucoidan-Chitosan Films*

Codes	Film Thickness (μm ± SD)	Water Vapor Permeability† (g ± SD)	Water Absorption Capacity‡ (g ± SD)	Tensile Strength (N ± SD)	Film Elongation (% ± SD)	Work of Bioadhesion (mJ/cm^2 ± SD)	Peak Detachment Force (mN/cm^2)
A1	29.7 ± 0.6	16.6 ± 0.8	0.67 ± 0.02	7.1 ± 0.7	36.3 ± 0.7	0.076 ± 0.006	405.0 ± 13.2
A2	41.3 ± 0.6	10.6 ± 0.5	0.83 ± 0.06	21.6 ± 0.3	6.7 ± 0.2	0.120 ± 0.007	605.0 ± 10.7
A3	51.7 ± 0.6	6.2 ± 0.2	1.03 ± 0.02	36.5 ± 1.9	8.0 ± 0.2	0.731 ± 0.010	2968.0 ± 17.3
A4	50.3 ± 0.2	6.3 ± 0.1	1.03 ± 0.02	20.7 ± 1.3	5.6 ± 0.1	0.722 ± 0.010	722.0 ± 20.1
B1	44.0 ± 0.1	5.7 ± 0.2	0.98 ± 0.01	45.8 ± 4.0	7.4 ± 0.3	0.181 ± 0.009	2521.0 ± 22.5
B2	58.3 ± 1.1	6.2 ± 0.1	1.09 ± 0.02	30.9 ± 1.0	9.5 ± 0.3	1.771 ± 0.030	5350.0 ± 17.3
C1	61.5 ± 0.8	6.3 ± 0.3	1.04 ± 0.04	14.6 ± 2.8	49.8 ± 2.1	1.158 ± 0.013	7162.0 ± 28.7
D1	64.0 ± 1.0	5.6 ± 0.2	0.96 ± 0.02	35.6 ± 0.6	5.5 ± 0.2	0.587 ± 0.012	5525.0 ± 16.5
D2	269.0 ± 3.0	3.3 ± 0.1	1.77 ± 0.04	12.2 ± 1.7	9.8 ± 0.1	1.136 ± 0.013	2647.0 ± 19.3

*SD indicates standard deviation.

†Water vapor permeability values of 0.1 g of the films.

‡Water absorption capacity values of 1.0 g of the films.

thinnest films showed the highest water vapor permeability and freeze-dried films showed the minimum water vapor permeability ($P < .05$). These data confirm the data found in the literature.[10,17,18] According to these findings, using films with optimum water vapor permeability in the treatment is advantageous because moisture and oxygen play an important role during wound healing.[18] During the study, this phenomenon was observed as the membrane got thinner and the fucoidan concentration increased.

The water absorption capacity of the films ranged from 0.67 to 1.77 g/g (Table 2). The same parameters that affected the water vapor permeability had an effect on the water absorption. Water absorption capacity increased with an increase in the ratio of chitosan/fucoidan concentration in the formulation ($P < .05$). The highest water absorption capacity in the formulations was that of freeze-dried films with 1.77 g/g ($P < .05$). This finding is considered to be related to the increasing pore size of lyophilized films.[10,17,18] However, clinical studies demonstrated that the films prepared by lyophilization completely absorb the wound fluid and prevent the formation of a medium with a moisture level suitable for the wound, thus complicating wound healing. In contrast, as stated in the literature, wound fluid should be absorbed in a balanced manner and moisture should be kept under control during wound healing.[2,3] Findings demonstrated that formulations prepared with the highest concentration of fucoidan (B2) and lactic acid (C1) have the ideal water absorption capacity and adding fucoidan to chitosan films increases the water absorption capacity of the membranes (Table 2).

Mechanical strength values of films were 7.1 to 45.8 N and elasticity values ranged from 5.5% to 49.8% (Table 2). All formulation parameters were found to be effective on mechanical strength of films ($P < .05$). Tensile strength of the films increased with increase in the ratio of chitosan/fucoidan concentration in the formulation ($P < .05$). Membrane with the highest mechanical strength was obtained by preparing a 0.25% concentration of fucoidan and rupture strength was 45.8 N. Strength of chitosan films was found to be increased with the addition of fucoidan to the formulation (A3-A4) ($P < .05$). In addition, tensile strength of films changed in inverse proportion to its elasticity; formulations prepared with 2% lactic acid concentration (C1) had the highest elasticity and lower mechanical strength (Figure 2) ($P < .05$). On the other hand, elasticity of the films decreased as chitosan concentration of the formulation increased. As stated in the studies by Khan et al[18] and Bangyekan et al,[19] while chitosan brings robustness to films, lactic acid provided elasticity, and this property was enhanced by increasing the concentration of polymer and the lactic acid used as solvent in the formulation. In addition, other polymer films used in combination with chitosan improved mechanical robustness of the films.[9,10,20] The study showed that increasing the fucoidan and lactic acid concentration in the formulation makes it easier to apply the films to the wound surface. Protection of the wound surface from external factors

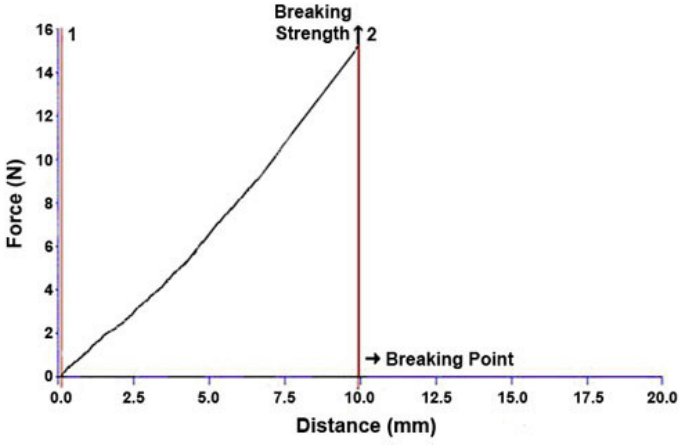

Figure 2. Tensile strength and elongation graph of C1 formulation (chitosan film containing fucoidan).

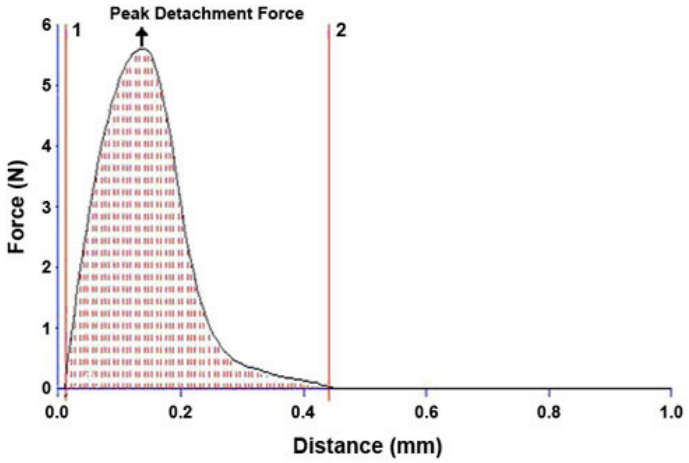

Figure 3. Work of bioadhesion graph of B2 formulation (highest concentration of fucoidan). Work of bioadhesion is area under the curve between 1 and 2 ($AUC_{1\text{-}2}$).

and elasticity of the dressing are the primary properties of ideal dressing material.[1,2,18] Therefore, the measured mechanical robustness of the membranes and the study findings demonstrated that adding of fucoidan to chitosan films increased the mechanical robustness (A4, B1) and elasticity (A4, B2) of the films, suggesting that the composite films with the select compositions reported here are superior compared with chitosan films.

The films' bioadhesion values ranged from 0.076 and 1.771 mJ/cm^2. All formulation factors were found to be effective on bioadhesion and the films' bioadhesive property was increased with increase of polymers and lactic acid concentrations in the membrane formulation ($P < .05$). The increase observed in the bioadhesive properties of the films as polymer concentration increased was considered likely to result from contact of the polymers with glycoprotein-rich mucous wound fluid, thus causing amine groups in the structure to combine with the negative charge groups (carboxyl, sulfate, etc) on the tissue surface; this property may enhance by increasing the chitosan concentration in the film.[20,21] Also, increased fucoidan concentration in the membrane is considered to cause reduction in the free amine groups of chitosan and thus to increase the amount of hydrophobic complex. Because of high cohesion, this increase prevents disintegration and rupture of the film in contact with the wound fluid, thus increasing the time of stay on the wound surface. This increased contact is important for efficient treatment on the wound site.[22,23] The findings of this study show that addition of higher concentrations of fucoidan and lactic acid increased bioadhesion and the formulations providing the optimum conditions were the ones prepared with 0.75% fucoidan (B2, 1.771 mJ/cm^2; Figure 3) and 2.0% lactic acid (C1, 1.158 mJ/cm^2). Evaluation of the data revealed that the films that were ideal in terms of thickness, physical and mechanical properties, and, most important, applicability were of C1 formulations, and these membranes are used for in vivo applications.

In Vivo Wound Healing Study

Macroscopic findings did not reveal any significant difference in terms of wound contraction area after day 7. Edema and increase in the polymorphonuclear leukocyte (PMNL) cells were only seen in control groups, and this increase of PMNL cells in control groups until day 14 demonstrated that the inflammation continues during this period, as shown in Table 3. Macroscopic observations on days 14 and 21 showed that wound contraction areas of groups treated with CFF are greater than other groups (Figure 4). Taken together, macroscopic findings suggest that wound healing levels can be ordered as CFF>CF>FS>control. The scheme of histopathological evaluation of the wound area is shown in Figure 5. There was no significant change between groups in the

Table 3. The Score of the Wound Cells and Collagen*

	Day 7				Day 14				Day 21			
Codes	Fibroblast	Collagen	MN	PMNL	Fibroblast	Collagen	MN	PMNL	Fibroblast	Collagen	MN	PMNL
Control	+ (6/6)	− (5/6)	− (5/6)	+ (2/6)	+ (4/6)	− (4/6)	− (4/6)	+ (3/6)	+ (4/6)	+ (3/6)	+ (6/6)	+ (5/6)
		+ (1/6)	+ (1/6)	++ (2/6)	++ (2/6)	+ (2/6)	+ (2/6)	++ (2/6)	++ (1/6)	++(3/6)		++ (1/6)
				+++(2/6)				+++ (1/6)	+++(1/6)			
FS	++ (6/6)	− (1/6)	− (5/6)	− (2/6)	+ (1/6)	+ (3/6)	− (2/6)	− (4/6)	+ (6/6)	+ (4/6)	− (4/6)	− (4/6)
		+ (5/6)	+ (1/6)	+ (4/6)	++ (5/6)	++ (3/6)	+ (4/6)	+ (2/6)		++ (2/6)	+ (2/6)	+ (2/6)
CF	+ (6/6)	− (4/6)	− (5/6)	+ (4/6)	+ (4/6)	+ (6/6)	− (4/6)	+ (4/6)	++ (5/6)	++ (4/6)	− (2/6)	− (3/6)
		+ (2/6)	+ (1/6)	++ (2/6)	++ (2/6)		+ (2/6)	++ (2/6)	+++(1/6)	+++(2/6)	+ (3/6)	+ (3/6)
											++ (1/6)	
CFF	+ (6/6)	+ (6/6)	− (3/6)	+ (3/6)	+ (1/6)	++ (4/6)	+ (3/6)	− (6/6)	− (4/6)	− (5/6)	− (6/6)	− (6/6)
			+ (2/6)	++ (3/6)	++ (1/6)	+++(2/6)	++ (2/6)		+ (2/6)	+ (1/6)		
			++(1/6)		+++(4/6)		+++(1/6)					

*MN indicates mononuclear leukocyte; PMNL, polymorphonuclear leukocyte; −, absent; +, mild; ++, moderate; +++, severe; FS, fucoidan solution; CF, chitosan film without fucoidan; and CFF, chitosan film containing fucoidan.

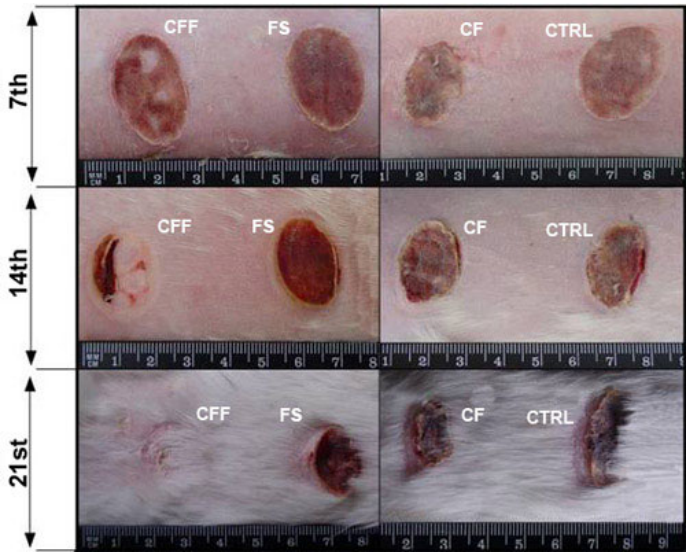

Figure 4. Photographs of the wound areas at 7, 14, and 21 days. CFF indicates chitosan film containing ficoidan; FS, fucoidan solution; CF, chitosan film without fucoidan; and CTRL, control.

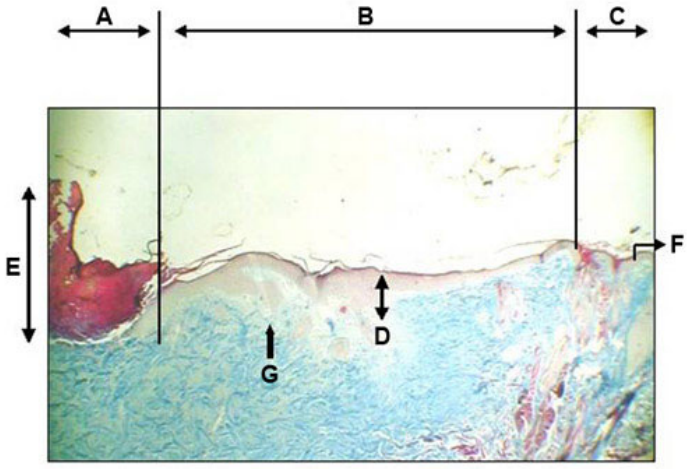

Figure 5. The scheme of the microscopic evaluation of the wound area: A, the eschar area; B, wound epithelium elongation; C, nonburned epithelium area; D, wound epithelium thickness; E, the eschar thickness; F, nonburned epithelium thickness; and G, papillary nick.

length of wound epithelium until day 7; however, the longest new epithelium formation was observed in the groups treated with CFF on days 14 and 21, as shown in Table 4 ($P < .001$). There was no statistically significant change in wound epithelial thickness on day 7; wound epithelial thickness of groups treated with FS and CFF increased compared with control group and group treated with CF, on day 14 (187 μm FS, 246 μm CFF). An increase in thickness was not seen in samples from the same groups on day 21. The reason for this increase in the wound epithelial length and thickness observed in CFF groups is considered to be the result of fibroblast population stimulant action owing to fucoidan affinity to fibroblasts[7,24] and increased efficacy through binding of some growth factors and cytokines, necessary for wound treatment, to fucoidan.[8] On the contrary, thickening of wound epithelium continued on CF-administered groups, on day 21 (Table 4). The increase related to fibroblast and collagen augmentation on the wound by day 14 was observed in groups treated with FS and CFF. The decrease in epithelium thickness on day 21 was considered to be the result of the higher healing rate particularly on wounds treated with CFF (Table 3). When considering the healing phase of burns started on day 7 to 14 and the heavy fibroblast migration into the wound area after day 7, collagen density increased on days 7 to 14; these data confirm in vitro/in vivo findings on the healing phase a documented in previous studies.[25,26] Also, macroscopic images of the wounds treated with CFF were parallel to histopathological findings. Groups treated with CFF were completely healed on day 21, while the healing of other groups was still in progress (Figure 4).

Papillary process is important for wound healing, ensuring that the new formed epithelium binds to dermis and sticks to tissue, as stated in the works of Erdağ and Sheridan.[27] As shown in Figure 6, papillary process was minimum in

Table 4. The Histopathological Values of the Wounds

	Wound Epithelium Elongation (μm ± SD)			Wound Epithelium Thickness (μm ± SD)			NOR Values (number ± SD)			Dermal Papillary Nick Values (number ± SD)		
Codes	7th	14th	21st	7th	14th	21st	7th	14th	21st	7th	14th	21st
Control	1302 ± 90	1950 ± 82	3533 ± 196	111 ± 10	154 ± 5	134 ± 8	2.69 ± 0.20	2.58 ± 0.20	2.63 ± 0.33	3.41 ± 0.47	3.94 ± 0.24	3.78 ± 0.39
FS	1455 ± 64	2086 ± 134	3586 ± 149	121 ± 8	187 ± 9	146 ± 10	2.94 ± 0.16	3.56 ± 0.53	3.53 ± 0.79	3.06 ± 0.41	6.11 ± 0.24	4.56 ± 0.65
CF	1391 ± 164	1833 ± 40	4050 ± 290	129 ± 13	148 ± 28	162 ± 5	3.00 ± 0	2.93 ± 0.15	4.63 ± 0.21	2.50 ± 0.43	3.50 ± 0.22	5.33 ± 0.42
CFF	1558 ± 105	2308 ± 47	4966 ± 292	114 ± 8	246 ± 24	169 ± 23	3.30 ± 0	7.03 ± 0.06	2.10 ± 0.10	2.83 ± 0.31	6.50 ± 0.67	7.50 ± 0.96

*NOR indicates nucleolar organizer regions; SD, standard deviation; FS, fucoidan solution; CF, chitosan film without fucoidan; and CFF, chitosan film containing fucoidan.

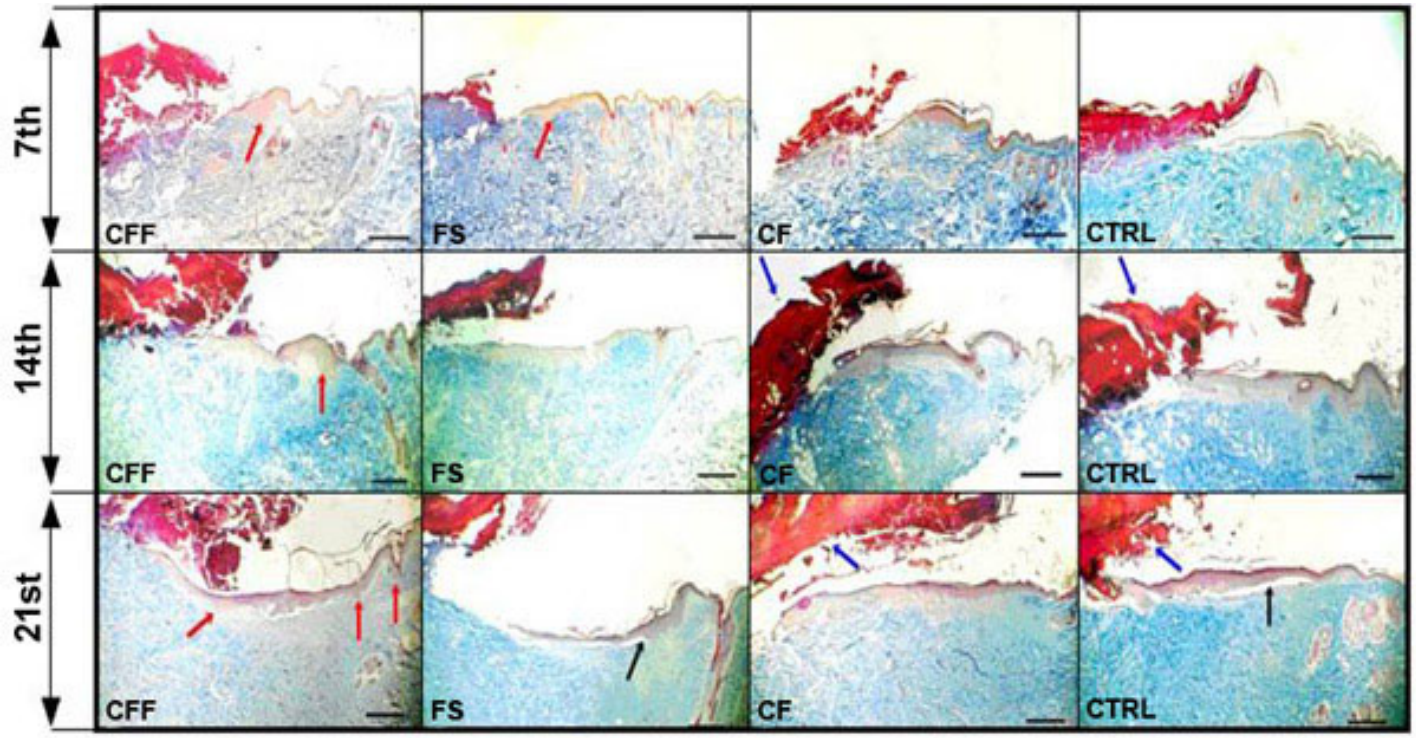

Figure 6. The histopathological photographs of the burn epithelial tissues stained with hematoxylin and eosin. Arrows: papillary nick (red); separation region between new epidermis and tissue (black); and scars (blue). Bars: 500 μm. CFF indicates chitosan film containing fucoidan; FS, fucoidan solution; CF, chitosan film without fucoidan, and CTRL, control.

the control group; while the maximum level of 7.50 was observed on day 14 in groups treated with CFF, with an increase by ~2.5-fold compared with CF groups (Table 4). Higher dissociation was observed in control groups having a weak, nonhomogenous papillary process beneath the new formed epithelium; however, there was no dissociation in other groups (Figure 6).

NORs are chromosomal segments that affect the cell proliferation, and the values indicating cell division activity[15,16] are listed in Table 4. In groups that were administered control, FS, and CF, NOR values increased depending on the healing process during treatment; however, NOR values reached the maximum level (7.03) in CFF groups and then decreased in the 14-day period ($P < .05$; Table 4). This change was considered due to higher growth of epithelium cells in groups treated with CFF, during the 7- to 14-day treatment period.

Conclusions

In conclusion, dermal burn healing experiments using rabbit model have shown that the application of fucoidan-chitosan film onto an open burn wound induces significant wound contraction, and accelerates the wound closure and healing process. Thus, the fucoidan-chitosan film may be a promising new dressing for wound occlusion and tissue repairing.

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