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APPLICATIONS OF MODIFIED CHITOSAN COMPOSITE MEMBRANES

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Abstract

The modified chitosan composite membranes (CASG-1 to CASG-4) were prepared with various ratios of chitosan, alginate, starch and glycerol by using casting and autoclaving methods. These membranes have smooth surfaces, highly transparent and pale yellow colour. The mechanical properties such as tensile strength, elongation at break and tear strength of these prepared membranes were determined. Based on the mechanical properties of the prepared composite membranes CASG-2 was chosen for biomedical applications. The antimicrobial activities of prepared membranes were tested by agar well diffusion method. The skin irritation test was conducted by Draize's method. The selected composite membranes (CASG-2) indicated that there is no irritation potential in albino rabbit skin. The selected composite membrane (CASG-2) was used to test in burn wound healing compared with standard sufre tulle drug. It was found that CASG-2 composite membrane was better than standard drug, sufre tulle for burn wound healing.

Keywords: composite membranes, mechanical properties, antimicrobial activities, skin irritation test, wound healing

Introduction

Higher molecular weight chitosan has been reported to have good membrane forming properties as a result of intra and intermolecular hydrogen bonding. The chitosan membrane characteristics, however, varied from one report to another due to their excellent properties and numerous applications like biocompatible coatings and membrane. Differences in the source of chitin used to produce chitosan, its properties, solvent used, method of membrane preparation, type of amount of copolymer, plasticizer used, affect the quality of chitosan membrane. These membranes have been studied in morphological aspects as well as in properties such as crystalline, porosity, and capacity of ion exchanger, etc. (Kurihara, 1994). Recently the formation of hybrid membranes of chitosan with inorganic networks has been also studied. The ability of chitosan membranes may permit its extensive use in the formation of membrane dosage forms or as drug delivery system. Chitosan could be dissolved in dilute organic acid such as lactic acid and acetic acid, prior to being cast into membranes. In order to assure for safety of biomedical application, skin irritation test should be carried out. This must be done to determine the risk of irritation due to the contact between the chemicals or formulations and human skin (More *et al*., 2013). Irritation is manifested by a tissue system in response to stimuli of either exogenous or endogenous origins (Carson *et al*., 1964). It has also been advocated as wound healing agent in the forms of a bandage. Its effect on wound healing in urogenital tissue has also been investigated (Nakatsuka *et al.,* 1992). In this work, composite membranes were tested on rabbit skin instead of human skin. Scoring system of rabbit skin irritation and response categories of irritation were shown in Table 1 (OECD TG 404, 2002) and Table 2 (Draize *et al*., 1944). Chitosan's unique properties make it useful for a broad variety of industrial and biomedical applications. In the present study, modified chitosan composite membranes were prepared and it may be used for medical purposes.

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Table 1 Scoring System of Rabbit Skin Irritation

Reaction	Score
Erythema	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar formation	4
Oedema	
No oedema	0
Very slight oedema	1
Well defined oedema	2
Moderate oedema (raising approximately 1mm)	3
Severe oedema (raising more than 1 mm and extended beyond the area of exposure)	4
Total possible score for primary irritation	**8**

OECD TG 404, 2002

Table 2 Response Categories of Irritation

Evaluations	Score
No Irritant	0.0
Negligible Irritant	0.1- 0.4
Slight Irritant	0.41-1.9
Moderate Irritant	2.0-4.9
Severe Irritant	5.0-8.0

Draize, Woodward & Calvery, 1944

Materials and Methods

Sample Collection

Chitosan sample was purchased from Shwe Poe Co. Ltd., Hlaing Tharyar Township, Yangon Region.

Starch was prepared from maize grain, *Zea mays* L. and this sample was procured from Insein Market, Yangon Region.

Preparation of Chitosan-Alginate-Starch-Glycerol (CASG) Composite Membranes

Modified chitosan composite membranes (CASG) were prepared by using 1.5 % (w/v) chitosan, 3 % (w/v) sodium alginate, 0.3 % (w/v) starch solution and various ratio of glycerol 0.05 %, 0.10 %, 0.15 % and 0.20 % and the prepared membranes were denoted as CASG-1, CASG-2, CASG-3 and CASG-4, respectively. The glycerol was also used as plasticizer for flexibility of membranes. The resulting modified composite solutions were autoclaved at a pressure of 0.1 MPa and 121 ± 1 ˚C for 1 h.

Mechanical Properties of Prepared Composite Membranes

Determination of thickness

Thickness of the prepared CASG-1 to CASG-4 composite membranes was measured by using NSK micrometer. The thickness of the membranes was measured at 5 points (center and 4 corners) using digital micrometer.

Determination of tensile strength and elongation at break

The prepared CASG-1 to CASG-4 composite membranes were cut off according to JIS K 7127 (1987) and the shape and dimension of test pieces were obtained. The both ends of test pieces were firmly clamped in the jaw of testing machine. One jaw was fixed and the other was moveable. The rate of moveable jaw was hold 100 mm/min. The resulting data was shown at the recorder. This procedure for tensile strength was repeated for three times.

Determination of tear strength

The specimen was cut off by using die-cutting. Specimen was cut with a single nick (0.05 mm) at the entire of the inner concave edge by a special cutting device using a razor blade. The clamping of the specimen in the jaw of test machine was aligned with travel direction of the grip in 100 mm/min. The order of the machine showed the highest force to tear from a specimen nicked. The procedure was repeated three times for each result.

Screening of Antimicrobial Activities of the Prepared Composite Membranes by Agar Well Diffusion Method

The prepared composite membranes: CASG-1 to CASG-4 were tested with *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and E. coli* species to investigate the nature of antimicrobial activity. After preparing the bacteriological media, the dried membranes were placed on the agar with flamed forceps and gently pressed down to ensure proper contact. The plates were incubated immediately or within 30 min after incubation or after overnight incubation at 37 ˚C.

Skin Irritation Test

Firstly, the albino rabbits were divided into two groups. Each group was included one rabbit. The 3 cm^2 area of hair from back quarter of each albino rabbit was shaved by using the shaver and cleaned by the clean water. The group-A used as control (with any treatment), and the group-B was treated with CASG-2 composite membrane respectively. The skin area of treated with CASG-2 albino rabbit was checked after 2 h later. If the area was shown redness, the animal was suffering the skin irritation. It observed redness with control to compare treated with CASG-2.

Animal experiment (burn wound healing)

Each Wistar albino rat was anaesthetized by injection Ketamine HCl and Xylazine (Anaesthesia) allowed for 30-45 min. Then the Ringer’s Lactate solution was induced by intraperitoneally to resist the heat effect. The hair from dorsal side of Wistar albino rat was shaved. A 150 g cylindrical metal rod (1 cm diameter) heated to 100 ºC in boiling water with an insulated rubber handle was used for the infliction of burns. Temperature was monitored using a thermometer. Burn infliction was limited to the loin area of all anaesthetized rats. The skin was pulled upwards, away from the underlying viscera, creating a flat surface. The rod was located on its own weight for 20 s on each rat. The average wound size was 1 cm in diameter.

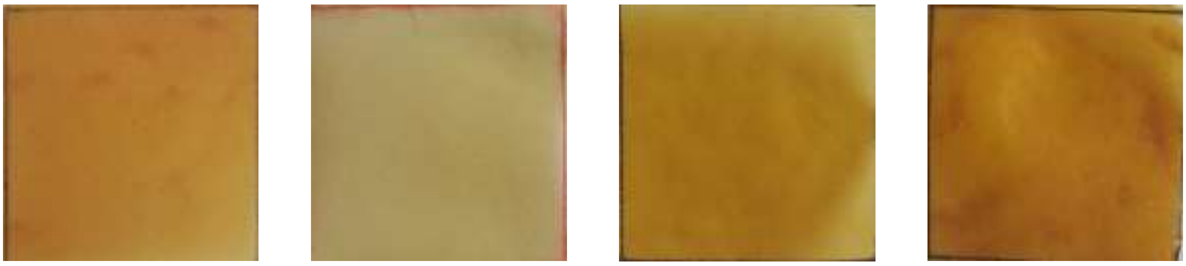
Histopathological finding

The skin lesions samples were obtained by necropsy was fixed with formalin for routine histopathological processing. Hematoxylin and eosin (H & E) stained and evaluated blinded manner by two observers using a light microscope with specific image analysis software from Olympus. For the morphological evaluation of skin lesions, a collagen fiber, inflammatory cell, blood vessel and granulation tissue of skin tissues were examined under a microscope by the pathologist.

Results and Discussion

Aspect of Membrane Preparation

In this preparation, modified chitosan-alginate-starch-glycerol composite membranes (CASG-1 to CASG-4) were prepared by using solvent casting and autoclaving method. These modified chitosan composite membranes (CASG-1 to CASG-4) showed smooth surface texture, transparent and pale yellow colour. These membranes to be employed as wound dressing, it should be durable, stress resistant, flexible, pliable and elastic.



(a) (b) (c) (d)

Figure 1 Modified composite membranes: (a) CASG-1 (b) CASG-2 (c) CASG-3 and (d) CASG-4

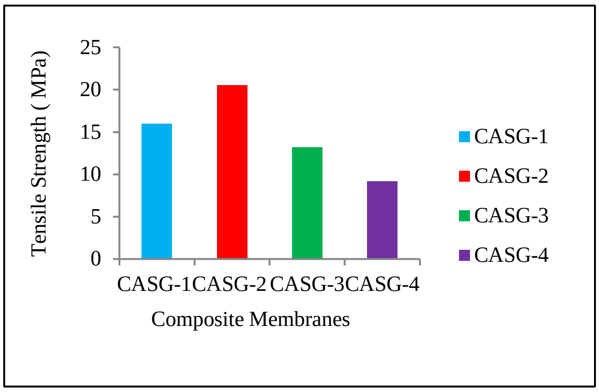
Mechanical Properties

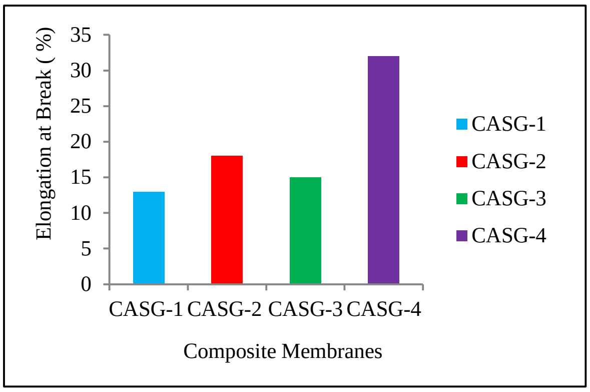
The mechanical properties such as tensile strength, tear strength and elongation at break are important parameters for showing the nature of membranes. The mechanical properties of CASG-1 to CASG-4 composite membranes are shown in Tables 3 and Figure 2. The more the tensile strength of membrane, the higher is the elasticity of the membrane. This means to point out that CASG-2 composite membrane are more flexible and more elastic.

Table 3 Mechanical Properties of the Modified Chitosan Composite Membranes

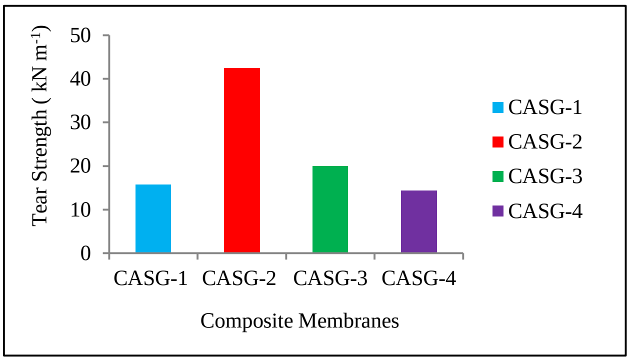
Membrane	Tensile strength (MPa)	Elongation at break (%)	Tear strength (kNm^{-1})
CASG- 1	16.00	13.00	15.70
CASG- 2	20.50	18.00	42.50
CASG- 3	13.20	15.00	20.00
CASG- 4	9.20	32.00	14.40

CASG- 1 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) +Glycerol (0.05 %) w/v
CASG- 2 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) +Glycerol (0.10 %) w/v
CASG- 3 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) +Glycerol (0.15 %) w/v
CASG- 4 = Chitosan (1.5 %) +Alginate (3.0 %) +Starch (0.3 %) +Glycerol (0.20 %) w/v
Thickness = ~ 0.10 mm





(a) **(b)**



(c)

Figure 2 Mechanical properties: (a) tensile strength (b) elongation at break and (c) tear strength of the chitosan composite membranes

Antimicrobial Activities of the Prepared Composite Membranes

Antimicrobial activities of the prepared CASG-1 to CASG-4 composite membranes were studied. These membranes were tested on six different strains of human pathogenic bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans*, and *E. coli*. To strengthen the finding, the antimicrobial activity of the all prepared membranes was determined by agar well diffusion method. Antimicrobial activities of the all prepared membranes were evaluated based on the diameters of clear inhibition zone surrounding the agar well. The prepared CASG-1 to CASG-4 composite membranes showed medium antimicrobial activities in the range of inhibition zone diameters (12 ~19 mm). Among them, the modified CASG-2 composite membrane showed that highest activity against six microorganisms (16 ~19 mm). The resulting data are shown in Table 4 and Figure 3.

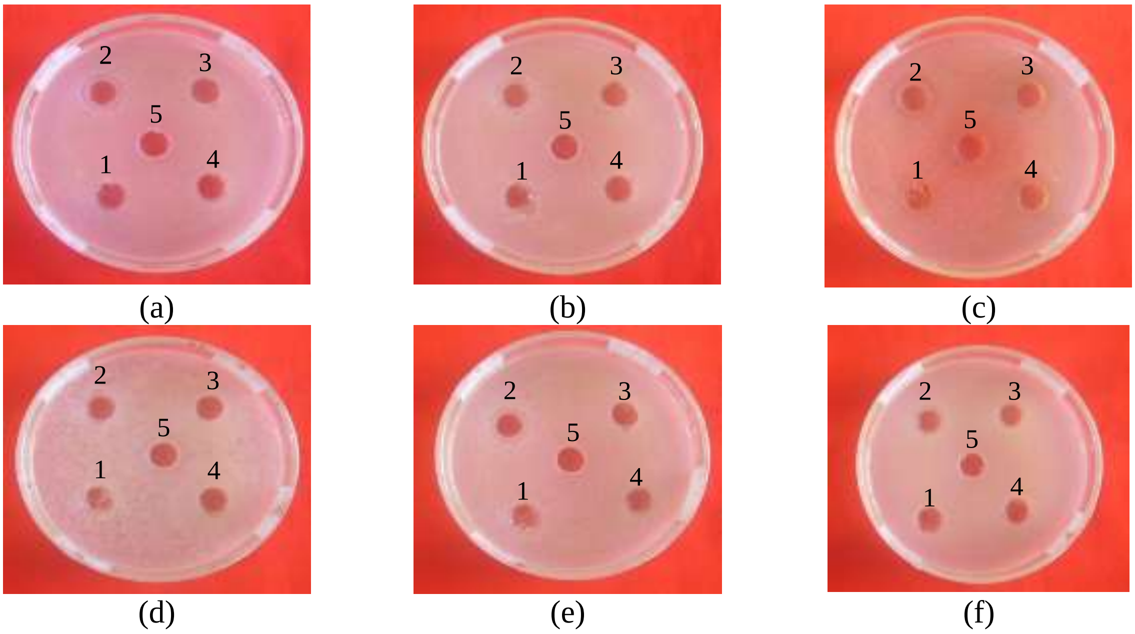


Figure 3 Antimicrobial activities of prepared chitosan composite membranes (1) CASG-1 (2) CASG-2 (3) CASG-3 (4) CASG-4 and (5) control

(a) *Bacillus subtilis* (d) *Bacillus pumilus*
(b) *Staphylococcus aureus* (e) *Candida albicans*
(c) *Pseudomonas aeruginosa* (f) *E. coli*

Table 4 Antimicrobial Activities of the Prepared Composite Membranes: CASG-1 to CASG-4

Membranes	Inhibition zone diameters (mm) of the samples against different organisms					
	(a)	(b)	(c)	(d)	(e)	(f)
Control	-	-	-	-	-	-
CASG-1	13(+)	15(++)	16(++)	14(+)	13(+)	16(++)
CASG-2	16(++)	17(++)	17(++)	16(++)	18(++)	19(++)
CASG-3	12(+)	15(++)	12(+)	13(+)	13(+)	16(++)
CASG-4	14(+)	16(++)	13(+)	14(+)	17(++)	18(++)

Ager well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

Organisms
(a) *Bacillus subtilis* (N.C.T.C-8236)
(b) *Staphylococcus aureus* (N.C.P.C-6371)
(c) *Pseudomonas aeruginosa* (6749)
(d) *Bacillus pumilus* (N.C.I.B-8982)
(e) *Candida albicans*
(f) *E. coli* (N.C.I.B-8134)

Skin Irritation Test

The irritant response was observed according to post-test observation periods. The animals were observed at 24, 48 and 72 h examination on these skin areas. Group-A rabbit (without treatment) showed no erythema and no oedema signs. Group-B rabbit (treated with CASG-2) composite membrane showed no erythema and oedema at 24, 48 and 72 h observation. Thus, it can be concluded that CASG-2 composite membrane has no irritation potential. These results were shown in Table 5 and Figure 4.

Score of Primary Irritation (SPI)

$$SPI = \sum \frac{Erythema\ and\ oedema\ grade\ at\ 24, 48\ and\ 72\ h}{Number\ of\ observation}$$

Primary Irritation Index (PII)

$$PII = \frac{\sum SPI\ (Test) - \sum SPI\ (Control)}{Number\ of\ animals}$$

Table 5 Score of Primary Irritation (SPI) and Primary Irritation Index (PII) for Rabbits (Control and Treated with Composite Membrane CASG-2)

Observation periods	Rabbit numbers (Group)					
	A (control)			B (CASG-2)		
	A_1	A_2	A_3	B_1	B_2	B_3
24 h (Erythema & Oedema score)	0	0	0	0	0	0
48 h (Erythema & Oedema score)	0	0	0	0	0	0
72 h (Erythema & Oedema score)	0	0	0	0	0	0
SPI		0			0	
PII		0			0	
Category		No irritant			No irritant	

* Draize *et al*., 1944 & OECD TG 404, 2002

(a)

(b)

First day Second day Third day

Figure 4 Physical appearances of albino rabbits skin test for (a) control and (b) treated with CASG-2 composite membrane

Appearance changes of burned skin

On days 5 and days 10, the wound area increased initially and the progress of all burned skins improvements were not significantly observed. On days 15, the crust was sloughed off completely in CASG-2 treated skin. On days 21, shaving was performed on all rats, since hair had grown extensively. The burn wound was almost completely healed that was treated with CASG-2.

From the observations, CASG-2 has been shown to significantly accelerate the burn wound healing. These results were shown in Figure 5.

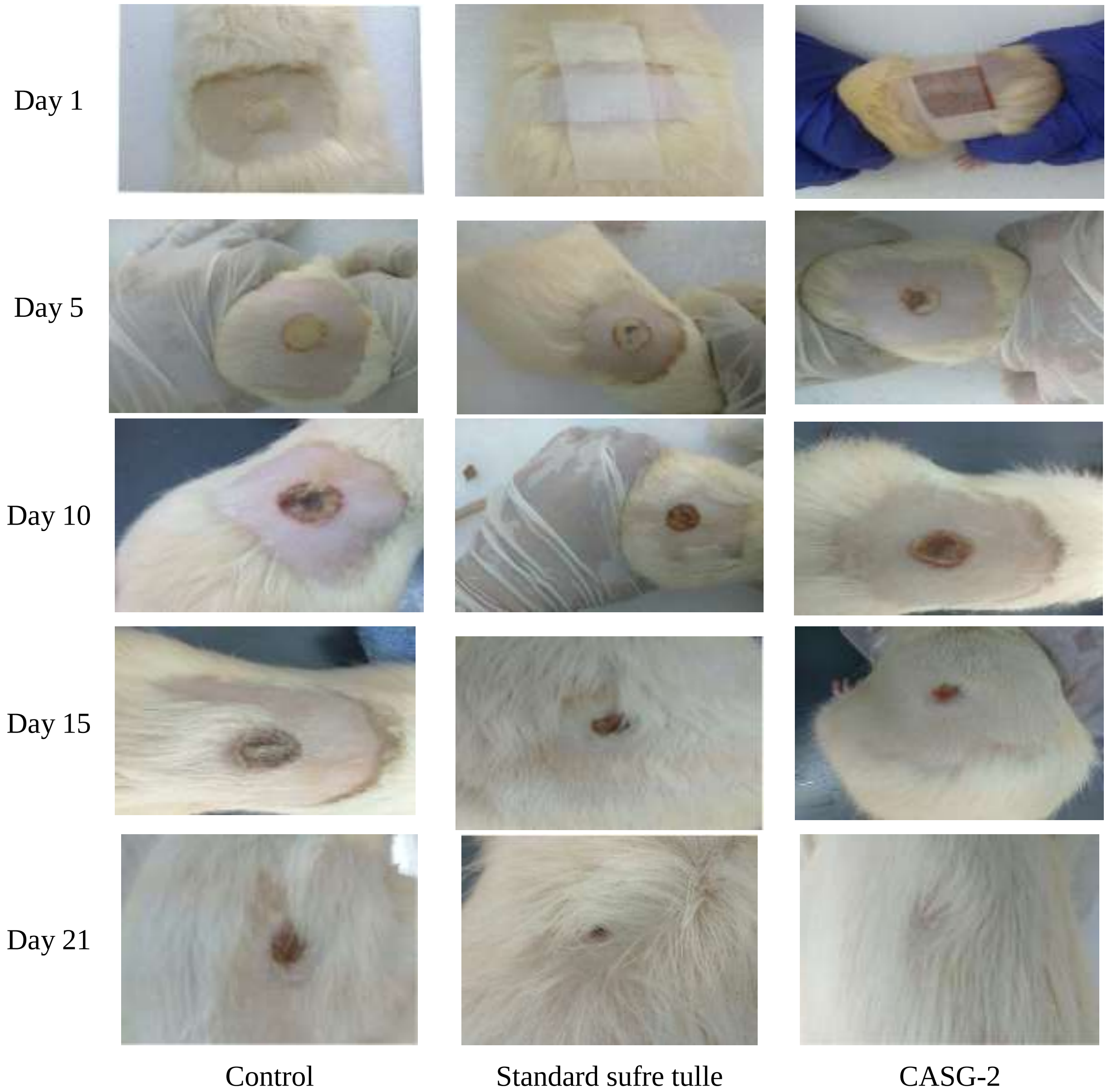
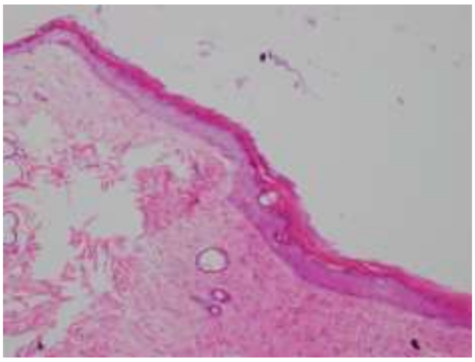


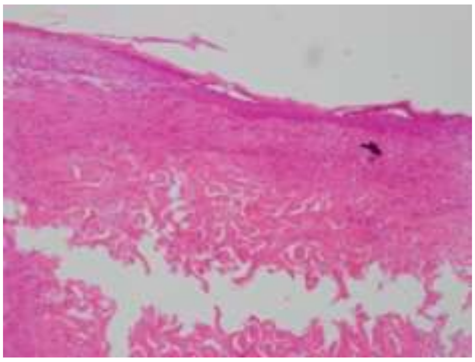
Figure 5 Treatment of burn skin by using control, standard sufre tulle and CASG-2 composite membranes

Histopathological examination

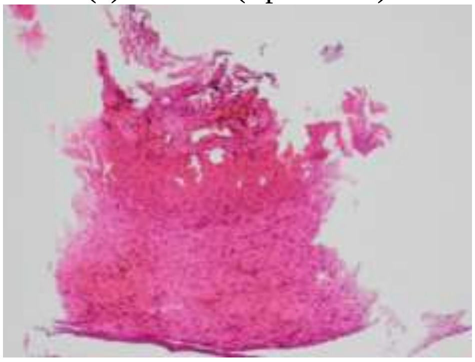
The crust layer of thin epidermis is detached from wound area. A few area of interrupted lining epithelium is still present in epidermis of control (no treatment) rat skin. The treated with sufre tulle (standard drug), there had a few sebaceous glands and sweat glands in epidermis layer without hair follicles. In the case of using CASG-2 as therapeutic agent of burned wound, well-developed sebaceous glands, sweat glands and hair follicles in epidermis and dermis layers of skin. Good wound healing in skin lesion of rat model.



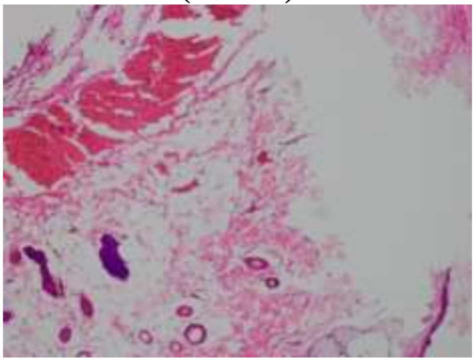
(a) Control (Epidermis)



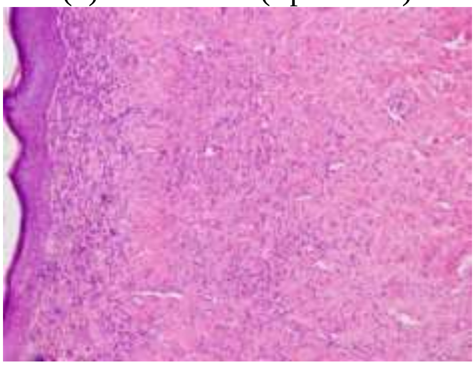
(Dermis)



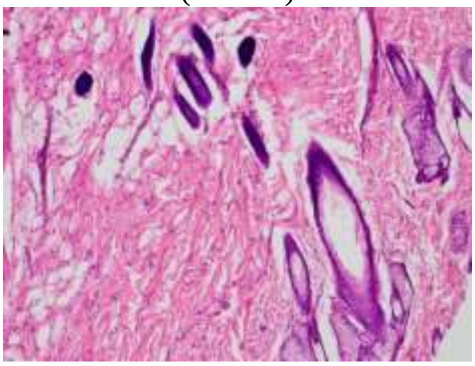
(b) Sufre Tulle (Epidermis)



(Dermis)



(c) CASG-2 (Epidermis)



(Dermis)

Figure 6 Histopathological examination (H& E) stained for morphological evaluation of burn skin (a) control, (b) treated with standard sufre tulle and (c) CASG-2 (after day 21) epidermis and dermis

Conclusion

In this study, polymer blended membranes consisting of chitosan, sodium alginate, starch and glycerol solution were prepared. The various ratios of modified chitosan-alginate-starch-glycerol (CASG-1 to CASG-4) composite membranes were prepared by solvent casting technique under autoclaving conditions at 121 ˚C and 0.1 MPa for 1 h. These modified composite membranes showed clear, smooth surface, flexible, highly transparent and light yellow colour. Based on the mechanical properties such as tensile strength, elongation at break and tear strength, the optimum condition was achieved by using 1.5 % (w/v) of chitosan, 3.0 % (w/v) of sodium alginate, 0.3 % (w/v) of starch solution, 0.1 % (w/v) of glycerol. According to mechanical properties, CASG-2 composite membrane was chosen as membrane to apply selected for biomedical applications. The antimicrobial activities of prepared CASG-1 to CASG-4 composite membranes were tested by agar well diffusion method. The prepared composite membranes gave antimicrobial activities, especially CASG-2 composite membrane indicated the highest activity on six microorganisms.

Skin irritation test showed that selected composite membrane: (CASG-2) was no irritation potential in albino rabbit skin. Based on the overall results, the selected composite membranes (CASG-2) are suitable to be used as wound healing and wound dressing for biomedical application.

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