Preparation and Characterization of Novel Sodium Alginate/Chitosan Two Ply Composite Membranes

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ABSTRACT: Sodium alginate/chitosan (SA/CS) two ply composite membranes were prepared by casting and solvent evaporation technique. NaHCO$_3$ was used as a porogen additive to form pores in the interior of the composite membranes and glycerol was introduced as a plasticizer. The water uptake capacity, mechanical strength, oxygen permeation property, and *in vitro* cytotoxicity were evaluated to test the feasibility to utilize the composite membranes for wound dressing. The average pore size, water uptake capacity, and oxygen permeation property of the composite membranes could be adjusted by the ratio of NaHCO$_3$ in the SA solution. The SA/CS two ply composite membranes showed high water uptake capacity, suitable mechanical strength, excellent oxygen permeability, and good biocompatible. It indicates that the SA/CS two ply composite membranes are suitable for wound dressing application. It provides a simple but promising platform to fabricate wound dressing using natural polymers. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 394–399, 2007

Key words: sodium alginate; chitosan; composite membrane; wound dressing; porogen additive

INTRODUCTION

To protect a skin defect from infections and dehydration, various wound dressings have been used more and more familiarly recently. Wound dressings are fabricated with various materials, such as alginate,[1–3] chitosan,[4] gelatin,[5] and poly(ethylene glycol).[6] Alginate, which is extracted from seaweeds, has been found to be extremely absorbent, be able to maintain a physiologically moist microenvironment that promotes healing and the formation of granulation tissue[1] and achieves hemostasis.[2] Chitosan (CS), composed of glucosamine and *N*-acetyl-glucosamine, is derived from chitin, which is the second most abundant natural biomaterial. It has been reported that chitosan could achieve hemostasis,[7] enhance the functions of inflammatory cells,[8–10] inhibit nitric oxide production that has been shown to contribute to cytotoxicity in cell proliferation during inflammation of wound healing by the activated RAW 264.7 macrophages[11] and allow the formation of granulation tissue with angiogenesis.[12] In the recent years, the complex of sodium alginate (SA) and chitosan (CS) has been used for drug delivery,[13] cell encapsulation,[14] pervaporation dehydration of isopropanol and ethanol,[15] and wound dressing.[16] In this study, novel SA/CS two ply composite membranes have been developed using a simple fabrication process. NaHCO$_3$ was used as a porogen additive to form pores in the interior of the composite membranes. The composite membrane has been characterized. The feasibility to utilize the composite membrane for wound dressing has been investigated.

MATERIALS AND METHODS

Materials

SA was obtained from Qingdao Jingyan Bio-Tech Development, China. The 2% (w/v) aqueous solution of SA exhibits a viscosity of 200 cp at 25°C. CS with a molecular weight of 3.0×10^5 and a deacetylation degree of 95% was purchased from Golden-Shell Biochemical, China. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT) and RPMI 1640 were purchased from Sigma Chemical, St. Louis. All the other chemicals used were of reagent grade and were used without any further purification.

Composite membrane fabrication

The SA/CS two ply composite membranes were fabricated by casting and solvent evaporation that was

described elsewhere[17] with modification. Briefly, SA and NaHCO$_3$ were dissolved in aqueous glycerol solution and stirred to obtain a homogenous mixture of 2% (w/v). The concentration of glycerol solution was 10, 20, and 30%, respectively. CS solution was prepared by dissolving 3.5% (w/v) CS in 2% (v/v) acetic acid. Both SA and CS solutions were filtered to remove any undissolved solids and impurities and left to stand until air bubbles have disappeared. At first, SA solution was cast onto a dry glass plate and allowed to dry at 40°C for 2 h. And then CS solution was cast onto the SA layer and spread on the surface of SA layer homogeneously. After drying at 40°C for 2 h, the glass plate was immersed into ethanol solution, and then the SA/CS composite membranes were detached from the glass plate gradually. The composite membrane was then subsequently immersed into 5% CaCl$_2$, 1% NaOH, and 5% CaCl$_2$ solution for 30 s each. After washed with a large amount of deionized water, the composite membrane was dried at vacuum condition for 24 h and stored in the desiccator. In this study, all measurements except mechanical property test were performed on the SA/CS composite membranes prepared with 10% glycerol solution.

Morphology

The surface morphology of the composite membrane was observed on a Phillp X130 Scanning electron microscope after the specimens were coated with an ultrathin layer of gold in a coating apparatus.

Water uptake capacity

To determine water uptake capacity, the known weight of composite membranes was immersed in phosphate buffer solution (PBS, pH 7.4) at room temperature for 24 h. The wet weight was determined using a 4-decimal-point microbalance after dabbing off the excess water on the surface of the sample. The percentage of water uptake of SA/CS composite membrane (E) was calculated from the formula:

$$E = [(W_w - W_d)/W_d] \times 100\%$$

where W_w and W_d are the wet and dry weight of the composite membrane, respectively. Each measurement was made in triplicate and the average was used to calculate the percentage of water uptake.

Mechanical property

The mechanical properties of the composite membrane were performed using Shimadzu AG-2000A universal strength tester at a crosshead speed of 5 mm/min. The test samples were cut into pieces in rectangular shape (30 × 7 mm^2). The thickness of the sample was measured using an Electronic Digital Micrometer (0–25 mm, 0.001 mm) at three points across the sample. The sample was held in place by the clippers, which were attached to the cell of the device. The upper clipper was driven upward to stretch the sample, and the tensile strength was monitored after the rupture of sample. The results were presented as the mean of five independent measurements.

Oxygen permeation property

Oxygen permeability through the composite membranes was performed using the gas permeability device described by Wu[18] with modification. The membrane was placed in the permeability chamber. The amount of oxygen to permeat through the sample was measured using a soap bubble flowmeter. The permeability coefficient P (cm^3 (STP) cm^{-2} s^{-1} cmHg^{-1}) was calculated from the following equation:

$$P = \frac{0.916 \, V_c}{\Delta t (p_f - p_p) A}$$

where p_f and p_p are the feed and permeate pressure (cmHg), respectively, V_c is the measured oxygen volume (cm^3), Δt is the testing time (s), A is the membrane area (cm^2).

Cytotoxicity tests

Extract medium from composite membrane was collected and was used to culture L929 cells followed by the measurement of cell viability using MTT assay. Briefly, samples with an area of 1.57 cm^2 were incubated in 15.7 mL RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C for 24 h in a humidified incubator with 5% CO$_2$ and 95% air.

L929 cells established from mouse connective tissue were inoculated in a 96 well culture plates at a density of 1×10^4 cells/well in 0.1 mL RPMI 1640 under a 5% CO$_2$ atmosphere and at 37°C for 24 h. After the cells attached to the plates, the medium was replaced with 0.1 mL extract medium. On day 2, 4, and 7 after the incubation under a 5% CO$_2$ atmosphere at 37°C, the medium was removed. About 0.98 mL RPMI 1640 and 0.02 mL MTT solution (5 mg/mL PBS) were added to each well and incubated for 4 h. Then the medium was aspirated and replaced with 0.15 mL DMSO to solubilize the MTT tetrazolium dye. The plates were shaken for 5 min and the optical density of the solution was read on a plate reader (Wellscan MK3, Labsystem, Finland) using a test wavelength of 570 nm and a reference wavelength of 630 nm. L929 cells with RPMI 1640 supplemented with 10% FBS served as control. All MTT assays were repeated four times. The cytotoxicity was expressed in the form of
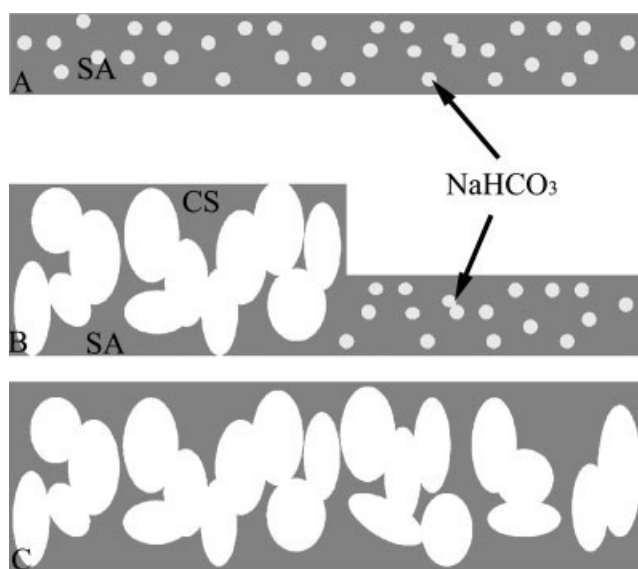
Figure 1 Preparation scheme of SA/CS two ply composite membrane by the casting and solvent evaporation technique.

the relative growth rate (RGR) using the following formula:

$$RGR(\%) = \frac{A_{test}}{A_{control}} \times 100$$

where A_{test} and $A_{control}$ are the average absorbance of the membrane and control, respectively.

RESULTS AND DISCUSSION

Composite membrane fabrication

In a previous study, Moon et al.[15] prepared SA and CS composite membranes using successive casting method. The results showed that membranes were two ply structure and dense. In this study, the SA/CS two ply composite membranes were fabricated by casting and solvent evaporation, where $NaHCO_3$ was introduced as a porogen additive. At first, SA solution was cast onto a dry glass plate [Fig. 1(A)]. When the CS solution was cast on the surface of SA [Fig. 1(B)], polyelectrolyte complex was formed through the ionic interaction between CS and SA in the interface. At the same time, $NaHCO_3$ previously mixed with the SA solution reacted with CH_3COOH, releasing CO_2 and H_2O, which were dissolved in the polymer matrix, leading to the formation of pores in the interior of the composite membranes[17] [Fig. 1(C)]. The thickness of the composite membranes was 105.4 ± 10.3 µm. The pore size is obviously dependent on the amount of $NaHCO_3$ (Fig. 2). The pore size increased with the increase in the amount of $NaHCO_3$ used. Figure 3 showed that the surface of the composite

membranes was dense and not smooth. The more $NaHCO_3$ was added, the more circular grooves appeared on the surface of the composite membranes.

Water uptake capacity

Water uptake capacity is one of the important parameters of wound dressing because wound dressing is supposed to absorb body fluid, which could lose from open wounds. To determine the feasibility of the SA/CS two ply composite membranes as wound dressing, the water uptake capacity of the composite membrane was measured. Both SA and CS are hydrophilic polymers and have excellent sorption capacity. When the composite membrane was immersed into PBS, SA and CS absorbed the water and started dilating. The percentage of water uptake of the dense composite membrane was 115%. The higher water uptake capacity could be attributed to the increase in the amount of $NaHCO_3$ (Fig. 4), which was resulted in the increase of void for adsorbing water. When the concentration of $NaHCO_3$ increased to 0.3%, the percentage of water uptake of the membrane could reach 740%.

Mechanical property

The mechanical property is another important factor to be considered in wound dressing. The composite membrane is expected to serve as a mechanical barrier, which undergoes stresses during use. The tensile strength and elongation rate at break were measured (Table I). It showed that the mechanical properties were correlated with the glycerol content of SA solution. SA and CS are both polar polymers, in which forces between molecular chains are strong. The composite membrane without glycerol was brittle. When
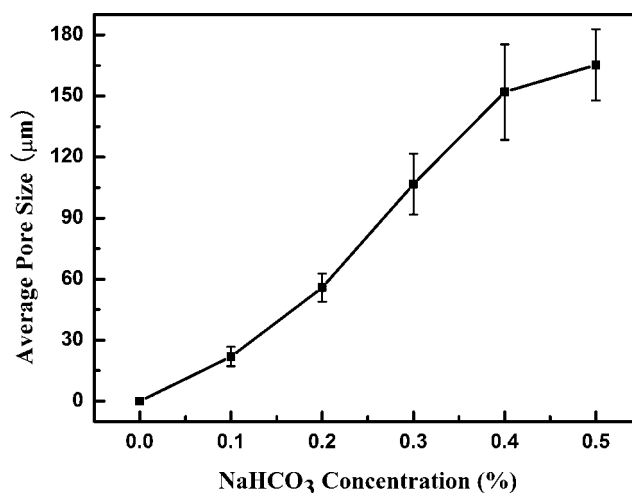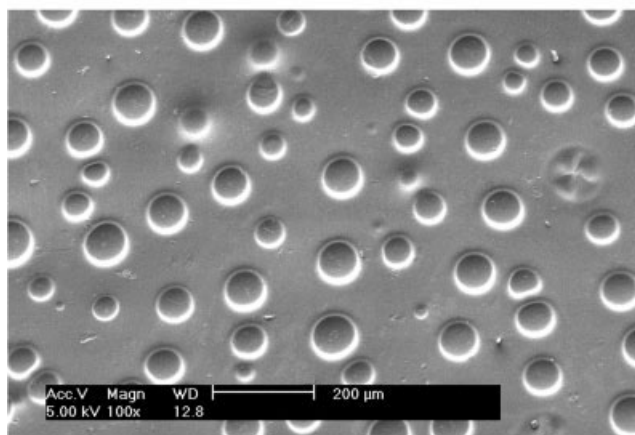

Figure 2 Average pore size of the composite membranes prepared with various $NaHCO_3$/SA ratios.
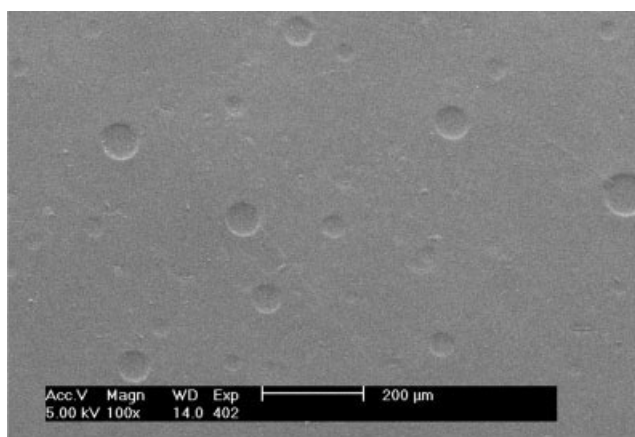
(a)
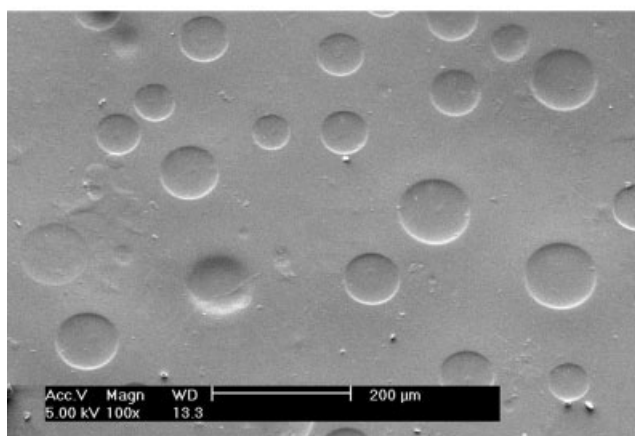
(b)

(c)

Figure 3 SEM micrographs of the composite membranes with various NaHCO$_3$ concentration: (A) 0.1%; (B) 0.2%; and (C) 0.6%.

the composite membrane was stretched by the clipper, it was ruptured with a little elongation. In this study, glycerol was introduced as a plasticizer to weaken forces between molecular chains. With the increase in glycerol content, the tensile strength of the composite membranes decreased and elongation rate
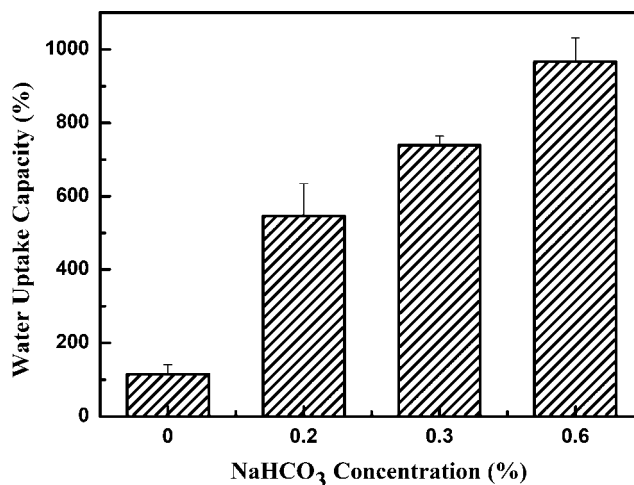
Figure 4 Water uptake capacity of the composite membranes prepared with various NaHCO$_3$/SA ratios.

at break increased. As shown in Table I, the tensile strength of the composite membrane was about 50 MPa and the elongation rate at break was less than 10%. When the glycerol concentration reached 20%, the tensile strength decreased to 2.50 MPa and the elongation rate at break increased to 32.68%. The tensile strength and elongation rate at break of wound dressing, which have been studied or used in clinic, were listed in Table II. When compared with these values, the strength of the two ply composite membranes fabricated by casting and solvent evaporation appeared to be sufficient for clinical applications and composite membranes exhibited favorable ductile under stress.

Oxygen permeation property

Oxygen permeation property is one of the important parameters for wound dressing as well. During wound healing, the cells require enough oxygen for their growth and metabolism. The oxygen permeation property of the two ply composite membranes was measured by a gas permeability device. Oxygen could barely permeate through dense SA/CS two ply composite membranes (Fig. 5). The solution-diffusion
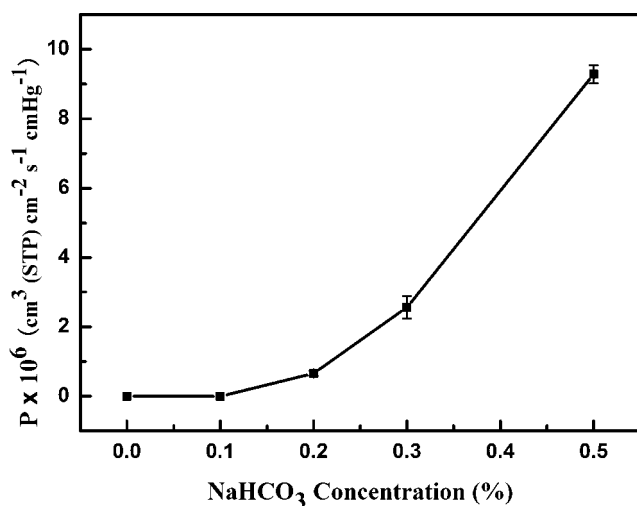
TABLE I
Tensile Strength and Elongation Rate at Break
of SA/CS Two Ply Composite Membranes
with Various Glycerol Contents

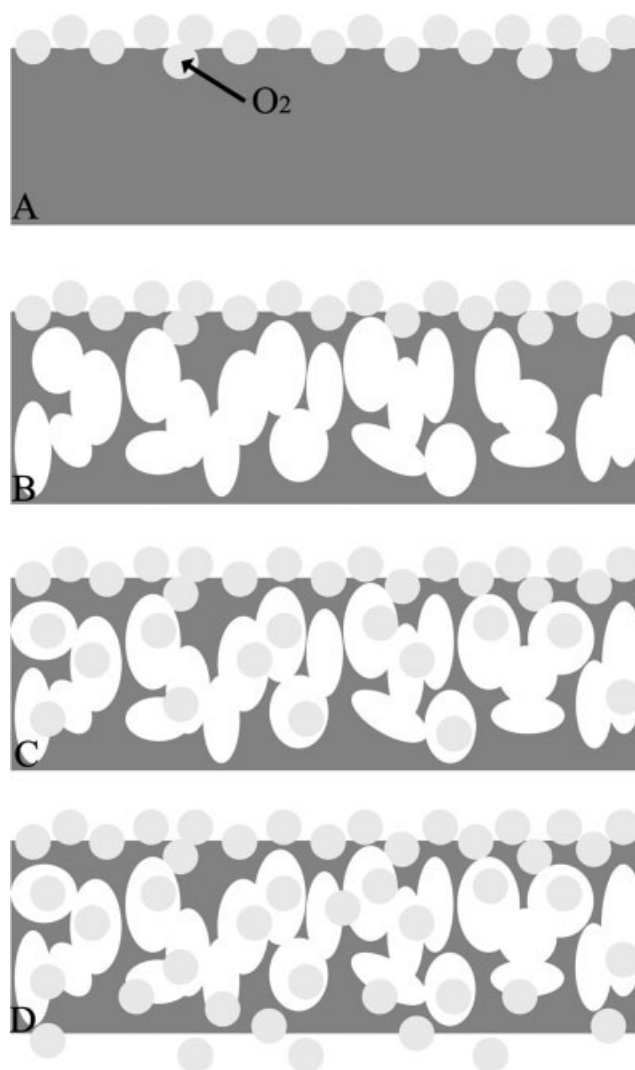| Glycerol concentration (%)	Tensile strength (MPa)	Elongation rate at break (%)
0	50.06 ± 3.51	7.86 ± 0.35
10	23.71 ± 2.54	15.49 ± 4.90
20	2.50 ± 0.79	32.68 ± 4.79
30	1.85 ± 0.53	40.69 ± 10.52

TABLE II
Tensile Strength and Elongation Rate at Break
of Several Kinds of Wound Dressings

Wound dressing	Tensile strength (MPa)	Elongation rate at break (%)
Chitosan/PVA[19]	26–94	8.7–170
Chitosan/gelatin[20]	6.5	20
II artifical skin[21] (collagen/PU)	1.99	56.2
41 artificial skin[21] (nylon/silicon)	2.04	88
PVP/PEG[6]	0.01–0.17	30–125
SA/CS	1.85–50.6	7.86–40.69

model, in which gas was first dissolved in the membrane material and then diffused through the membrane down a concentration gradient,[22] was employed to illustrate oxygen permeation. When oxygen reached the composite membane, it was dissolved into the surface layer of the composite membrane. Because of the high membrane density and the strong molecule interaction between SA and CS, oxygen could barely diffuse through the composite membrane any more [Fig. 6(A)]. Pores being generated in the interior of the composite membrane improved oxygen permeability significantly. The permeation process of oxygen through the composite membrane was depicted in Figure 6(B–D). After adsorbed by the surface layer of the composite membrane [Fig. 6(B)], oxygen entered into the pores [Fig. 6(C)]. Because the pore size was extremely larger than the mean free path of oxygen, the permeation process followed viscous flow model and the oxygen transfer resistance in the composite membrane was negligible. When oxygen reached the underlay of the composite membrane, oxygen was adsorbed by membrane and desorbed under pressure [Fig. 6(D)]. The permeation of


Figure 6 The permeation process of oxygen through the composite membrane.

oxygen through the composite membrane should be dominated by the dense part. The increase in $NaHCO_3$ significantly decreased the thickness of the dense part, therefore increased the oxygen flux. The results suggested that the SA/CS two ply composite membranes showed excellent oxygen permeability and could provide controlled oxygen permeation, which can fit the basic requirements for a membrane to be used as wound dressing.

Cytotoxicity tests

According to the Regulation for the Supervision and Administration of Medical Devices,[23] the cytotoxicity of the SA/CS two ply composite membranes was determined by the MTT assay. The results were given in Figure 7. It was calculated that RGR values for 2, 4, and 7 days were 99.63, 82.66, and 89.28%, respectively. Since the cytotoxicity was estimated as first

Figure 5 Permeability coefficient of the composite membranes prepared with various $NaHCO_3$/SA ratios.
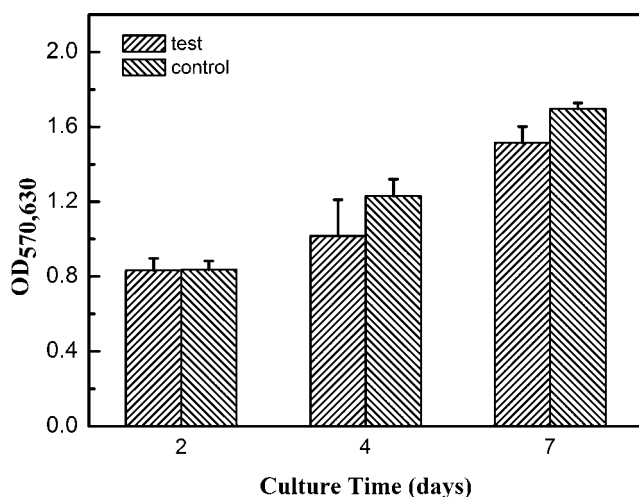
Figure 7 Cytotoxicity of the composite membrane based on L929 viability.

degree when RGR value was more than 80%, it demonstrated that the composite membrane was biocompatible and had no toxic effect as wound dressing.

CONCLUSIONS

In this study, SA/CS two ply composite membranes were fabricated by casting and solvent evaporation. The preparation process was simple and easy to carry out. The composite membranes demonstrated high water uptake capacity, suitable mechanical strength, excellent oxygen permeation property, and good biocompatibility required for wound dressing. It is feasible to utilize the SA/CS two ply composite membranes for wound dressing.

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