Antibacterial Activity of Chitosan–Alginate Sponges Incorporating Silver Sulfadiazine: Effect of Ladder-Loop Transition of Interpolyelectrolyte Complex and Ionic Crosslinking on the Antibiotic Release

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ABSTRACT: Hydrogel membranes prepared from polyelectrolyte complex (PEC) have been used for repair of wounds and controlled antibacterial release. A simple method, based on homogenizing interpolyelectrolyte complex, has been developed to prepare a chitosan–alginate sponge with high stability. The spongelike chitosan–alginate hydrogel can be used as a wound dressing for the sustained release of silver sulfadiazine (AgSD) in a controlled way. In this study, we evaluated the effect of electrolyteic properties of chitosan and alginate on the characteristics of the prepared chitosan–alginate PEC. All types of the spongelike chitosan–alginate hydrogels exhibited superabsorbent properties. However, only the chitosan–alginate hydrogel prepared by the interpolyelectrolyte complex of alginate with low pH of chitosan, and that prepared by the interpolyelectrolyte complex of chitosan with high pH of alginate, can keep their stability after swelling in PBS solution. FTIR analysis suggests that the protonated amino groups on chitosan and the ionized carboxylic groups on alginate should be responsible for the formation of a stable ladder-type of chitosan–alginate PEC. Ionic crosslinking is helpful to increase the stability of the loop-type of chitosan–alginate PEC. The release of AgSD from chitosan–alginate PEC sponges could be controlled by the variation of ladder-loop structural transition of chitosan–alginate PEC and the ionic crosslinking of the chitosan–alginate complex. The antibacterial ability of AgSD-incorporated PEC sponges was examined in agar plate against *Pseudomonas aeruginosa and Staphylococcus aureus.* The result suggests that the PEC sponges containing antimicrobial agents should effectively suppress bacterial proliferation to protect the wound from bacterial invasion. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 538–549, 2005

Key words: chitosan–alginate polyelectrolyte complex (PEC); wound dressing; polysaccharides; biopolymers; hydrogels

INTRODUCTION

Hydrogels, prepared from interpolyelectrolyte complex reactions, have attracted considerable interest because of the great variety of biomedical applications.[1–4] Hydrogels produced from biopolymers, such as polysaccharides, have been proposed for the design of wound healing materials.[5–7] The control of wound healing using the hydrogel-based wound dressing has many advantages, such as allowing drainage of wound exudate and providing a substantially wet environment to prevent dehydration of injured skin.[8–10]

Chitosan and alginate are both polysaccharides derived from marine biopolymers, the formulas of which are shown in Figure 1. Chitosan is a copolymer of glucosamine and *N*-acetylglucosamine obtained by N-deacetylation of chitin. Alginate is an acidic linear polysaccharide composed of two sugars, L-guluronic acid and D-mannuronic acid, in variable proportions. The amino group of chitosan is easily protonated at moderately low pH (<6.3) to form cationic polyelectrolyte, and the carboxylic acid group of alginate is easily ionized in water to form anionic polyelectrolyte. The polycation and polyanion, carrying electrostatically complementary ionizable groups, can form a stable polyelectrolyte complex. Capsules or beads prepared from chitosan–alginate polyelectrolyte complex have gained considerable attention in controlled-delivery systems for macromolecular drugs, such as in-

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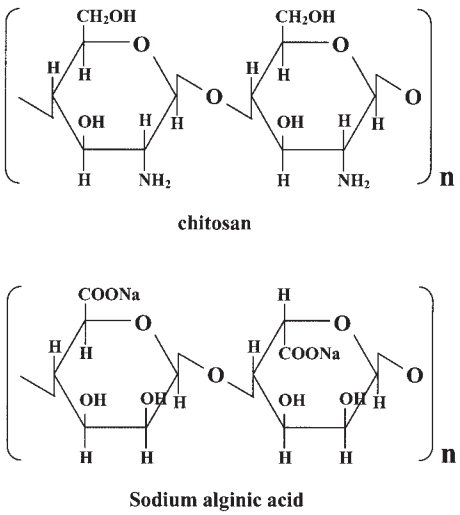

Figure 1 Schematic molecular structures of chitosan and alginate.

suline, hirudin, and albumin.[11–14] It was recognized that the chitosan layer could increase the stability of alginate capsules and control the diffusion rates of the encapsulated materials.

Chitosan has many desirable characteristics such as biocompatibility, enzymatic degradability, and antibacterial activity.[15,16] It also has an acceleratory effect on the wound healing process.[17–20] Alginate can be converted into a hydrophilic gel that provides a moist wound environment to promote wound healing and epidermal regeneration.[21,22] For these reasons, chitosan- or alginate-based materials were investigated for their possible uses as wound healing materials.[23–26] Some researchers reported the fabrication of polyelectrolyte complex (PEC) composed of chitosan and alginate for wound dressing application.[27,28] The chitosan–alginate PEC wound dressing was first prepared by freeze-drying chitosan solution, followed by putting the dried chitosan sponge into an alginate aqueous solution and then freeze-drying it to form a chitosan–alginate PEC sponge. The PEC sponge was sequentially put into chitosan and alginate solutions, alternatively, for a complete interpolyelectrolyte complex.[27] An improved method for preparation of the chitosan–alginate PEC membranes was developed by reacting chitosan with sodium alginate in an aqueous medium containing acetone. It was found that the effect of chitosan molecular weight on the properties of the prepared chitosan–alginate PEC films was significant.[28,29]

The objective of the present investigation was to offer a simple method for the preparation of a macroporous chitosan–alginate PEC membrane as a wound dressing with sustainable antimicrobial ability. By using a homogenizing interpolyelectrolyte complex method, different types of chitosan–alginate PEC membranes could be fabricated, depending on the preparation conditions. The chitosan/alginate blend ratio, the protonation of chitosan and the ionization of alginate for interpolyelectrolyte complex, and the ionic crosslinking of the chitosan–alginate complex with calcium ion (Ca^{2+}) as control factors were examined to understand these effects on the reactions of polyelectrolyte complex and physical properties of prepared PEC membranes (such as swelling capability and porous structure). Recently, the prevention of bacterial infection became an important factor required for successful wound healing; therefore, several antibiotic-impregnated wound dressings were developed to meet the requirements.[11,30,31] In this study, silver sulfadiazine (AgSD) as an antimicrobial agent was incorporated into the spongelike PEC membrane to deter wound infection. This article reports the sustainable antibiotic delivery ability and *in vitro* antimicrobial efficiency against *Pseudomonas aeruginosa* and *Staphylococcus aureus* investigated in this study.

EXPERIMENTAL

Materials

Chitosan was purchased from Fluka Chemie (Buchs, Switzerland) and sodium alginic acid salt was purchased from Lancaster Synthesis (Lancashire, UK). Sliver sulfadiazine (AgSD) was obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents and solvents used were of reagent grade.

Preparation of chitosan–alginate PEC membrane

The chitosan–alginate PEC membrane was prepared by using a homogenizing interpolyelectrolyte complex method. Chitosan solution (1.0% by weight) was prepared by dissolving chitosan powder (2 g) in 200 mL of deionized water containing acetic acid (1.0% by weight) at room temperature. Alginate solution (1.0% by weight) was prepared by dissolving sodium alginate powder (2 g) in 200 mL of deionized water at room temperature. The dissolved chitosan solution was then added into the alginate solution and blended with an IKA T25 homogenizer (IKA® Works, Inc., Wilmington, NC) until an opaque aqueous solution was obtained. The solution was sonicated to remove the trapped air bubbles. The air bubble–free solution was poured into a glass disk in a dust-free atmosphere to be lyophilized by an FD-5N freeze-drier (Eyela, Tokyo, Japan) for the preparation of chitosan–alginate PEC membrane. Blend ratio, pH values of chitosan and alginate solution, and the time of ionic crosslinking were used as variable factors to prepare chitosan–alginate PEC membranes at different conditions. The chitosan–alginate blend ratios by weight were 1/5, 1/1, and 5/1 (series I: CA15, CA11, and CA51), respec-

tively. Chitosan solutions at pH 1, 2, and 4 were blended with alginate solution at a constant pH value (pH 7) to prepare PEC membranes (series II: C1A7, C2A7, and C4A7). Conversely, different pH values (pH 4, 7, and 10) of alginate solution were blended with chitosan solution at a constant pH value (pH 7) to prepare PEC membranes (series III: C4A4, C4A7, and C4A10).

Ionic crosslinking of chitosan–alginate PEC membranes

The homogenized chitosan–alginate blend was cast onto a glass disk for interpolyelectrolyte complex as described above. The next step was to immerse in calcium sulfate aqueous solution (0.1 wt %) for 10, 60, and 360 min. The membrane, crosslinked by calcium ions, was washed with deionized water and freeze-dried to prepare ionically crosslinked chitosan–alginate PEC membrane (series IV: CA0, CA10, CA60, and CA360).

Characterization of chitosan–alginate interpolyelectrolyte complex

The characteristic absorption of chitosan–alginate interpolyelectrolyte complex, which ranged from 400 to 4000 cm^{-1}, was recorded on a model FTIR-408plus FTIR spectrophotometer (Jasco, Tokyo, Japan).

Scanning electronic microscopy (SEM)

The chitosan–alginate PEC membranes prepared by the homogenizing interpolyelectrolyte complex method were attached onto double-sided adhesive tape and fixed to an aluminum stage, respectively. The membranes were cut by a razor, then were sputter-coated with gold to a thickness of 500×10^{-8} cm using an IB-2 coating unit (Hitachi, Osaka, Japan). Subsequently, the morphologies of cross section and both sides of the membranes were examined by use of a Hitachi S-2300 scanning electron microscope.

Swelling ability

The swelling ratio of each chitosan–alginate PEC membrane was determined by swelling the membranes in the physiological buffer saline (PBS) at room temperature. The chitosan–alginate PEC membrane (200 mg) was placed in the PBS solution for a required period of time. Subsequently, the swollen chitosan–alginate PEC membrane was taken out and the wet weight of the chitosan–alginate PEC membrane was determined by first blotting the membrane with a filter paper to remove the adsorbed water on the surface, then by weighing the membrane immediately on an electronic balance. The percentage swelling of chitosan–alginate PEC membrane in the medium was calculated as follows:

$$E_{st} = [W_t - W_0)/W_0] \times 100$$

where E_{st} is the percentage swelling of chitosan–alginate PEC membrane at a predetermined time. W_t denotes the swollen weight of the chitosan–alginate PEC membrane at a predetermined time and W_0 is the initial weight of the chitosan–alginate PEC membrane. Each swelling experiment was repeated three times and the average value was taken as the percentage swelling value.

Water vapor evaporation

The rate of water vapor evaporation was determined according to ASTM method E96-90, Procedure D. An evaporimeter was constructed in a closed glass chamber to prevent variations arising from ambient conditions. The apparatus consisted of a glass chamber with a cover, isothermally bathed at 35°C, and a digital hygrometer with a continuous percentage of relative humidity (RH), temperature, and dew point display. Saturated magnesium chloride solution was placed in the glass chamber to maintain RH at 40 ± 1%. A permeability cup was filled with 20 g of deionized distilled water, and the test PEC membrane was fixed onto its opening. Evaporation of water through the test membrane was monitored by measurement of loss of weight of the cup. An open cup was used as the control.

Antibiotic-release studies

Drug release studies using a 1.5 mm diameter silver sulfadiazine incorporated PEC membrane were conducted in a vertical permeation cell to simulate local *in vivo* release onto a burn-wound area. The permeation cell, consisting of donor and receptor compartments, was maintained at 35°C by circulating thermostated water through the water jacket. The antibiotic incorporated PEC membrane was mounted between the donor and receptor compartments, and the receptor compartments, filled with PBS solution, were stirred continuously at 200 rpm. At predetermined time intervals, 0.5 mL of the dissolution medium was taken out from the sampling mouth and replaced with fresh PBS solution after sampling. The dissolution medium was examined by UV spectrophotometer at 236 nm to determine the concentration of dissolved sulfadiazine.

In vitro antibacterial assay

The inhibition zone of the AgSD-incorporated chitosan–alginate PEC membrane (5 mm diameter discs) was measured on agar plates inoculated with *P.*

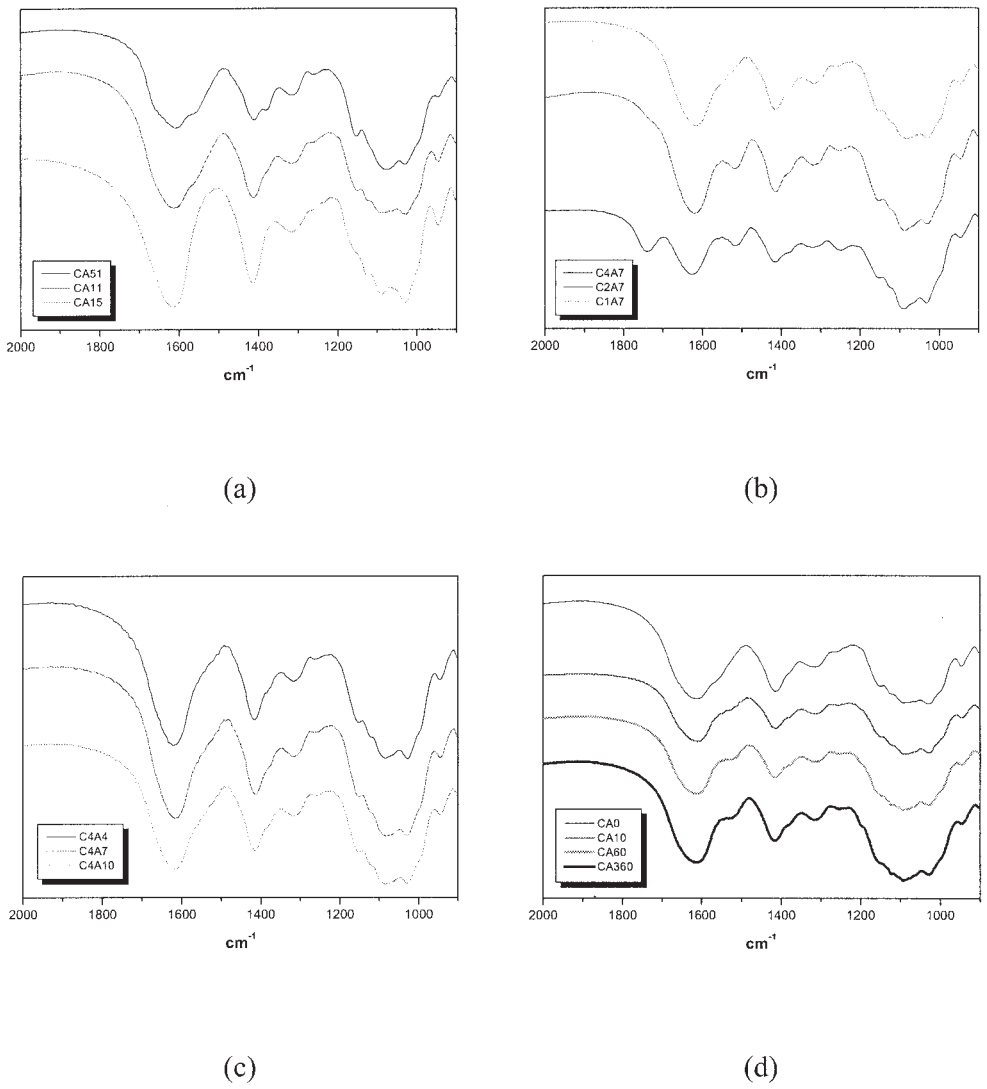

Figure 2 ATR-FTIR spectra of various chitosan–alginate PEC membranes. Type of PEC membrane: (a) chitosan–alginate blend ratios are 5/1, 1/1, and 1/5 (series I: CA15, CA11 and CA51); (b) blend chitosan solution (pH 1, 2, and 4) with pH 7 of alginate solution (series II: C1A7, C2A7, and C4A7); (c) blend alginate solution (pH 4, 7, and 10) with pH 4 of chitosan solution (series III: C4A4, C4A7, and C4A10); (d) ionic crosslinking for 0, 10, 60, and 360 min (series IV: CA0, CA10, CA60, and CA360).

aeruginosa strain and *S. aureus* strain using the inhibition zone test. AgSD is released from the chitosan–alginate PEC membrane into inoculation medium to inhibit the growth of *P. aeruginosa* strain and *S. aureus* strain. After 24 h incubation, inhibition zones (diameter of inhibitory circle) around the AgSD-incorporated chitosan–alginate PEC membrane were compared with those of blank PEC membrane without silver sulfadiazine formulation.

RESULTS AND DISCUSSION

Characterization of chitosan–alginate interpolyelectrolyte complex

The positively charged chitosan and negatively charged alginate can form interpolyelectrolyte complex by electrostatic attraction. To examine the chemical structures of various chitosan–alginate PEC membranes prepared under different interpolyelectrolyte complex conditions, the PEC membranes were examined by FTIR spectra. Figure 2(a) shows the FTIR spectrum of PEC membranes prepared from different chitosan–alginate blend ratios. The characteristic peak at around 1560 cm^{-1}, with respect to the protonated amino groups ($–NH_3^+$) of chitosan, was overlapped by the characteristic peak at around 1620 and 1450 cm^{-1}, with respect to the carboxylic ion groups of alginate ($–COO^-$). The intensity of characteristic absorption of $–NH_3^+$ decreased with the increase of chitosan/alginate blend ratio, accompanied with the increased intensity of characteristic absorption of $–COO^-$. Figure 2(b) shows the FTIR spectra of PEC membranes pre-

pared by, respectively, blending chitosan solutions of different pH values (pH 1, 2, and 4) with alginate solution at constant pH value (pH 7). The PEC membrane prepared from low pH value of chitosan solution (pH 1 and pH 2) and the alginate solution shows the appearance of new peaks at around 1741 cm^{-1} (C1A7) and 1516 cm^{-1} (C1A7 and C2A7), attributed to carbonyl (C═O) stretching of free carboxylic acid and protonated amino ($–NH_3^+$) groups. Conversely, the FTIR spectra of PEC membranes, prepared by blending alginate solutions of different pH values (pH 4, 7, and 10) with chitosan solution at constant pH value (pH 4), are shown in Figure 2(c). Chemical structures of the prepared chitosan–alginate PEC membranes do not show signs of obvious change with the variation of pH value of alginate solution. Figure 2(d) shows the ionic crosslinking of chitosan–alginate PEC membranes with calcium ion (Ca^{2+}). The strength of the protonated amino ($–NH_3^+$) group at 1516 cm^{-1} increases with increasing time for ionic crosslinking, suggesting that the degrees of interpolyelectrolyte complex decrease with increasing degree of ionic crosslinking.

We suggest that carboxylic ion groups ($–COO^-$) of alginate substantially react with protonated amino ($–NH_3^+$) groups by electrostatic attraction, to form interpolyelectrolyte complex, and the residual carboxylic ion groups were reduced to free carboxylic acid groups. However, the chemical structures of the prepared chitosan–alginate PEC membranes do not change obviously with the variation of pH value of alginate solution. Calcium ions were found to be in competition with protonated amino group of chitosan to bind with carboxylic ion groups of alginate, leading to the inhibition of chitosan–alginate interpolyelectrolyte complex.

Morphology of chitosan–alginate hydrogel membranes

Chitosan has a pK_a value of 6.5, whereas alginate has pK_a values of 3.4 to 4.4. Under certain circumstances, the amino groups in chitosan are protonated and the carboxyl groups in alginate are ionized. The positively charged chitosan and negatively charged alginate can form interpolyelectrolyte complex by electrostatic attraction. Direct homogenizing of chitosan and alginate solution leads to development into PEC networks and the formation of unsoluble gel. The removal of inert water within the networks by a freeze-drying process results in the formation of a homogeneous superporous structure. Ionic crosslinking with Ca^{2+} reduces the pore size of the PEC membranes arising from the decrease of inert water within the networks. The spongelike matrix can promote the drainage of exudates and the preparation of an optimum wound bed for autografting. Adsorption of water in the superporous chitosan–alginate PEC hydrogel also provides a substantially wet environment in which epidermal regeneration can be promoted.

SEM micrographs of the chitosan–alginate PEC membranes are shown in Figure 3. This figure shows that the morphological structures of these PEC membranes were composed of homogeneous macropores (200–400 μm). As shown in Figure 3, the different interpolyelectrolyte complex conditions (pH of chitosan and alginate solution, and ionic crosslinking) used in this work for tailoring various chitosan–alginate PEC membranes have a perceptible influence on the porous structures of membranes. This is especially significant for the PEC membrane ionically crosslinked with Ca^{2+}. The pore size decreased from 400 to 100 μm with increasing crosslinking time, from 10 to 360 min. The results are explained as follows: the inert water within the networks that could be removed by freeze-drying was decreased with increasing crosslinking time.

Swelling and stability of chitosan–alginate PEC membranes

Figure 4(a) shows the swelling behavior of PEC membranes prepared from different chitosan/alginate blend ratios. The membrane reaches the maximum swelling ratio at 10 min postoperation, followed by gradual loss of its weight. The rate of weight loss increases with decreasing chitosan/alginate blend ratio. The excessive alginate, which is unreacted (by interpolyelectrolyte complex) with the protonated chitosan, will be dissolved from the membrane into PBS solution and is responsible for the weight loss of the chitosan–alginate PEC membrane under swelling in PBS solution. Figure 4(b) shows the swelling behavior of PEC membranes prepared by blending chitosan solutions with different pH values with alginate solution at constant pH value (pH 7). The PEC membranes prepared from chitosan solutions of pH 2 and pH 4 (C2A7 and C4A7) lose their weight after reaching the maximum swelling ratio; however, the PEC membrane prepared from chitosan solution of pH 1 (C1A7) swells at stable condition with the increase of its weight. This result indicates that the PEC membrane prepared by the interpolyelectrolyte complex of alginate with a chitosan solution of low pH value (pH 1) is more stable than that prepared by the interpolyelectrolyte complex of alginate with a chitosan solution of high pH value (pH 2 and pH 4). Figure 4(c) shows the swelling behavior of PEC membranes prepared by blending alginate solutions of different pH values with a chitosan solution at constant pH value (pH 4). The weight loss of PEC membrane decreases with increasing pH value of alginate solution. Figure 4(d) shows the swelling behavior of the ionic crosslinked PEC membranes. It is found that weight lost after

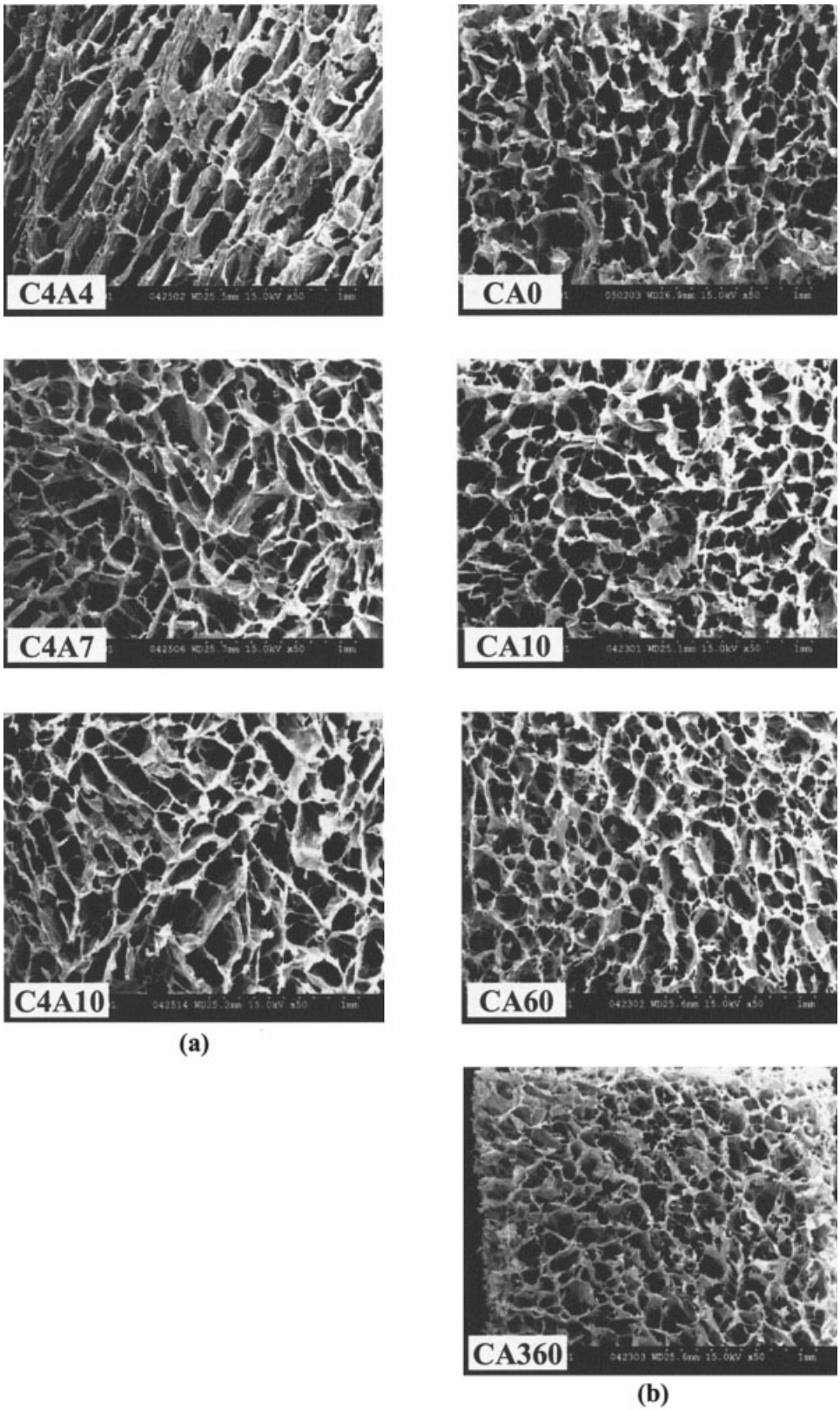

Figure 3 SEM micrographs of various chitosan–alginate PEC membranes. Type of PEC membrane: (a) blend alginate solution (pH 4, 7, and 10) with pH 4 of chitosan solution (series III: C4A4, C4A7, and C4A10); (b) ionic crosslinking for 0, 10, 60, and 360 min (series IV: CA0, CA10, CA60, and CA360).

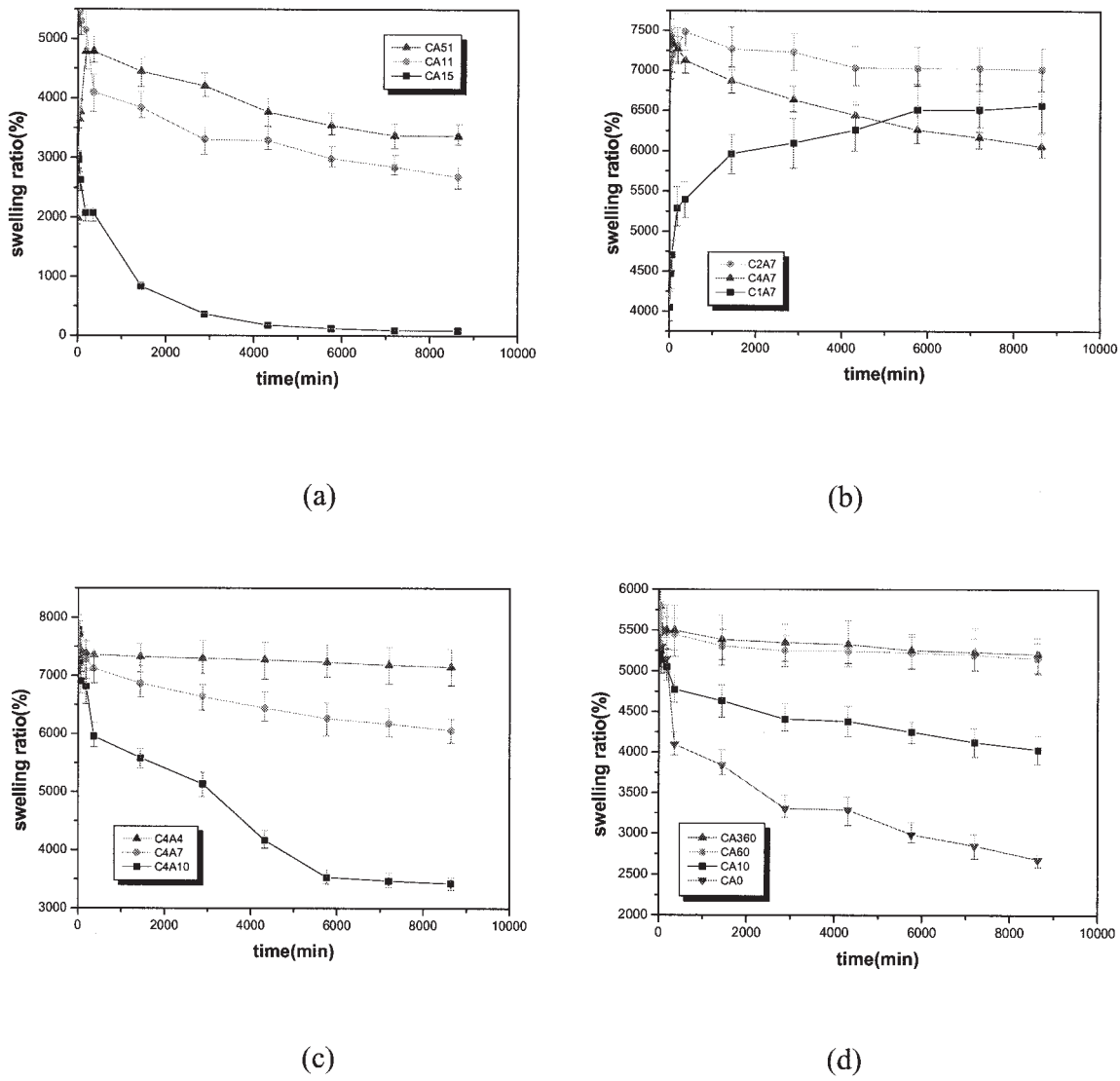

Figure 4 Swelling capability of various chitosan–alginate PEC membranes. Type of PEC membrane: (a) chitosan–alginate blend ratios are 5/1, 1/1, and 1/5 (series I: CA15, CA11, and CA51); (b) blend chitosan solution (pH 1, 2, and 4) with pH 7 of alginate solution (series II: C1A7, C2A7, and C4A7); (c) blend alginate solution (pH 4, 7, and 10) with pH 4 of chitosan solution (series III: C4A4, C4A7, and C4A10); (d) ionic crosslinking for 0, 10, 60, and 360 min (series IV: CA0, CA10, CA60, and CA360).

long-term swelling of the chitosan–alginate PEC membrane can be reduced by ionic crosslinking. The chitosan–alginate PEC membrane reaches equilibrium swelling without an obvious weight loss after crosslinking with Ca^{2+} for 60 min.

All of the PEC membranes exhibit a rapid swelling rate and superabsorbent properties. The superabsorbent properties of these homogeneous PEC membranes are attributed to their large capacity (macropores in the membrane), allowing the membranes to absorb a great quantity of water. The increased stability of the PEC membrane is attributed to the more complete interpolyelectrolyte complex and the conversion of ionized carboxylic groups of alginate into free carboxylic acid, which reduces the solubility of prepared PEC networks. Figure 5 shows the schematic loop-ladder structural transition of chitosan–alginate polyelectrolyte complex. It is suggested that ladder-type of the chitosan–alginate PEC membrane, prepared from an alginate solution of high pH value (pH 10), is more stable than the loop-type of chitosan–alginate PEC membrane, prepared from an alginate solution of low pH value (pH 7 and pH 4).

According to the loop-ladder theory, one can conclude that a more complete reaction to form the ladder-type of interpolyelectrolyte complex is achieved by the interaction of a chitosan solution at pH 4 with an alginate solution at pH 10 rather than an alginate

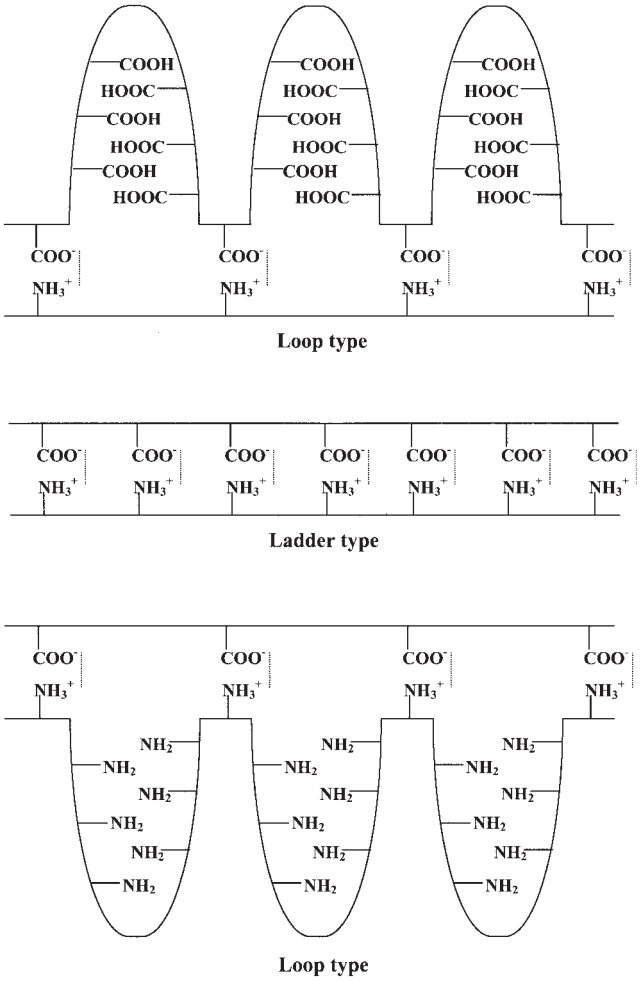

Figure 5 Schematic loop-ladder type of chitosan–alginate interpolyelectrolyte complex.

solution at pH 7 or pH 4. Similarly, a more complete reaction to form the ladder-type of interpolyelectrolyte complex is achieved by the interaction of an alginate solution at pH 7 with a chitosan solution at pH 1 rather than a chitosan solution at pH 2 or pH 4 [Fig. 4(b)]. The increased electrostatic linkages between the $-NH_3^+$ group on chitosans and $-COO^-$ groups on alginate decrease the solubility of PEC membrane in PBS solution. Weight loss of the chitosan–alginate PEC membrane after long-term swelling can be reduced by ionic crosslinking. The fixation of chitosan–alginate complex networks, by the crosslinking of interpenetrated macromolecular chains of alginate with Ca^{2+} ions, is responsible for the increased stability of the chitosan–alginate PEC membranes.

Release of silver sulfadiazine

Figure 6 shows the total amount of AgSD released from a chitosan–alginate PEC membrane against different conditions for interpolyelectrolyte complex. Figure 6(a) shows the drug release behavior of PEC membranes prepared from different chitosan–alginate blend ratios. The total AgSD released from chitosan–alginate PEC membrane decreased with increasing content of alginate in the membrane. These observed phenomena could be explained by the increasing hydrophilicity of the PEC membrane with increasing alginate content. AgSD was released from the PEC membrane accompanied by the dissolution of uncomplex alginate. Figure 6(b) shows the drug release behavior of PEC membranes prepared by the complex of chitosan solutions of different pH values with alginate solution at a constant pH value (pH 7). The PEC membranes prepared from chitosan solutions at pH 2 and pH 4 (C2A7 and C4A7) demonstrate a significantly quicker AgSD release rate, compared to that of PEC membrane prepared from a chitosan solution at pH 1 (C1A7). Figure 6(c) shows the drug release behavior of PEC membranes prepared by blending alginate solutions of different pH values with a chitosan solution at constant pH value (pH 4). There is no obvious difference of drug release between the PEC membranes prepared from alginate solutions of different pH values (pH 4, 7, and 10). Figure 6(d) shows the drug release behavior of chitosan–alginate PEC membranes crosslinked with Ca^{2+} ions. It is found that over 90% of incorporated AgSD was released from the chitosan–alginate PEC membrane without crosslinking with Ca^{2+} ions; however, only 60 and 50% of incorporated AgSD was released after crosslinking with Ca^{2+} ions for 60 and 360 min, respectively. This suggests that the drug release rate of the chitosan–alginate PEC membrane is reduced by ionic crosslinking.

It is interesting to find that the pH values of chitosan for the preparation of chitosan–alginate PEC membrane indeed affect the AgSD release; however, the pH values of alginate show an insignificant effect on drug release. These results indicated that the pH level of chitosan might be a dominating factor to influence the release of AgSD from the chitosan–alginate PEC membrane. In fact, a protonated amine function group would be helpful to create an electrostatic attraction force between sulfadiazine and chitosan, leading to reducing the release rate of AgSD. The drug release rate of the chitosan–alginate PEC membrane is effectively reduced by ionic crosslinking. The fixation of chitosan–alginate networks by ionic crosslinking with (Ca^{2+}) ions decreases the hydrophilicity of the PEC membrane, which is responsible for the reduced drug release rate.

In vitro antibacterial test

The antibacterial ability of the chitosan–alginate PEC membrane loaded with AgSD was examined by the determination of inhibition zone around the disk of

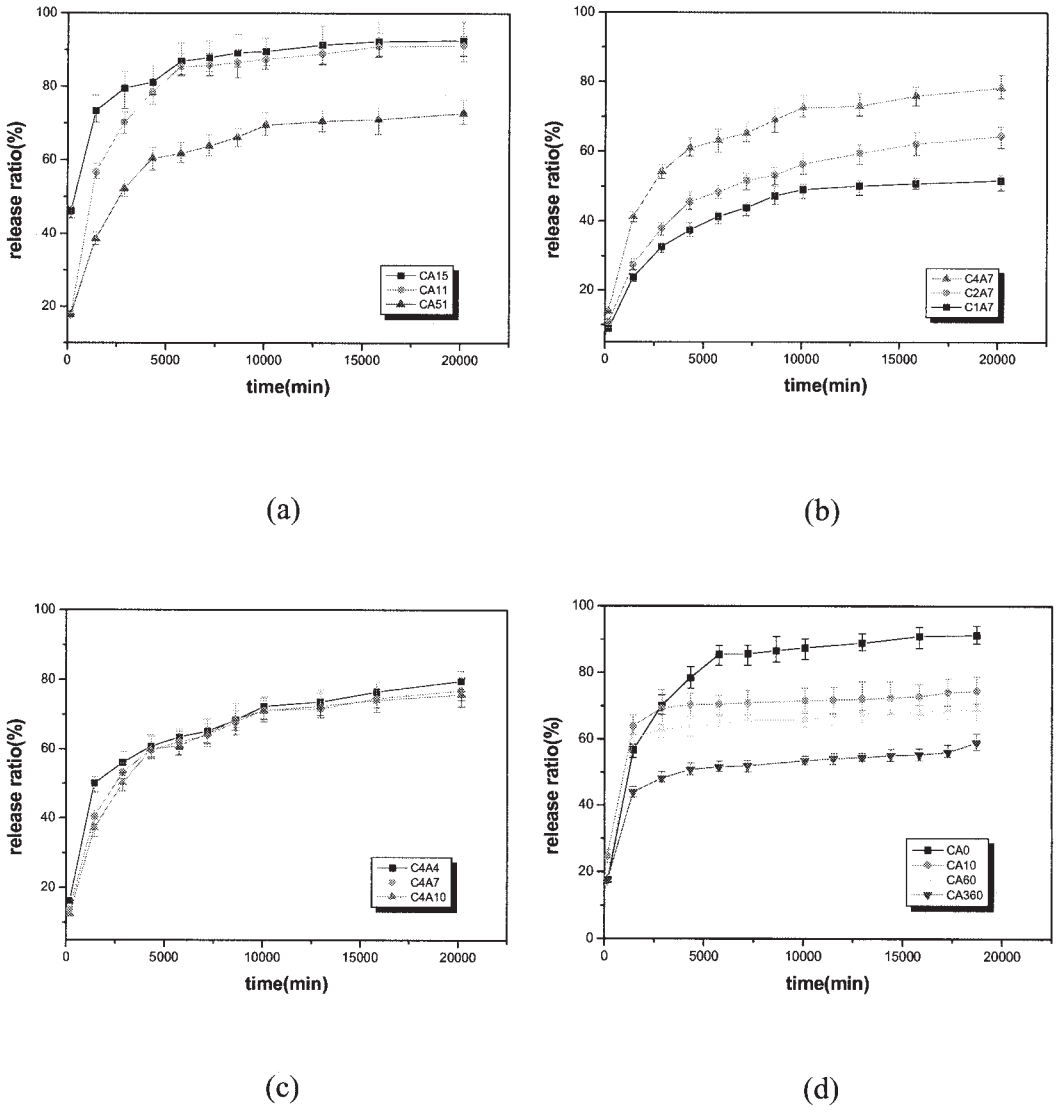

Figure 6 AgSD release from various chitosan–alginate PEC membranes. Type of PEC membrane: (a) chitosan–alginate blend ratios are 5/1, 1/1, and 1/5 (series I: CA15, CA11, and CA51); (b) blend chitosan solution (pH 1, 2, and 4) with pH 7 of alginate solution (series II: C1A7, C2A7, and C4A7); (c) blend alginate solution (pH 4, 7, and 10) with pH 4 of chitosan solution (series III: C4A4, C4A7, and C4A10); (d) ionic crosslinking for 0, 10, 60, and 360 min (series IV: CA0, CA10, CA60, and CA360).

PEC membranes. Three types of PEC membranes (C1A7, CA11, and CA15) incorporated with AgSD were used for the antibacterial assay. A blank chitosan–alginate PEC membrane was used as the control group. Figure 7 shows the photographic image of three types of PEC membranes and control group on culture plates inoculated with *P. aeruginosa* and *S. aureus*, respectively. The inhibition zone is apparent from culture plates incubated with the PEC membranes, except for control group. The summarized culture time versus inhibition zone is shown in Figure 8. The results reveal that the antimicrobial capability of AgSD-incorporated chitosan–alginate PEC membranes against *P. aeruginosa* was more effective than that against *S. aureus*. It was also found that the diameter for the inhibition zone of the CA15 membrane remained at 16 mm without reduction after 7 days of incubation. However, the diameters for the inhibition zones of the C1A7 and CA11 membranes decreased to about 10 mm after the same period of incubation. The largest inhibition zone of CA15 suggests that this membrane has the most effective antimicrobial capability against *S. aureus* and *P. aeruginosa* among the three types of PEC membranes.

P. aeruginosa is known as a Gram-negative bacteria and *S. aureus* is a Gram-positive bacteria. The cell wall of Gram-negative bacteria is a thinner structure with distinct layers. Gram-positive bacteria have thicker cell walls compared to those of Gram-negative bacteria. Sulfadiazine blocks the combination of *P*-amino-

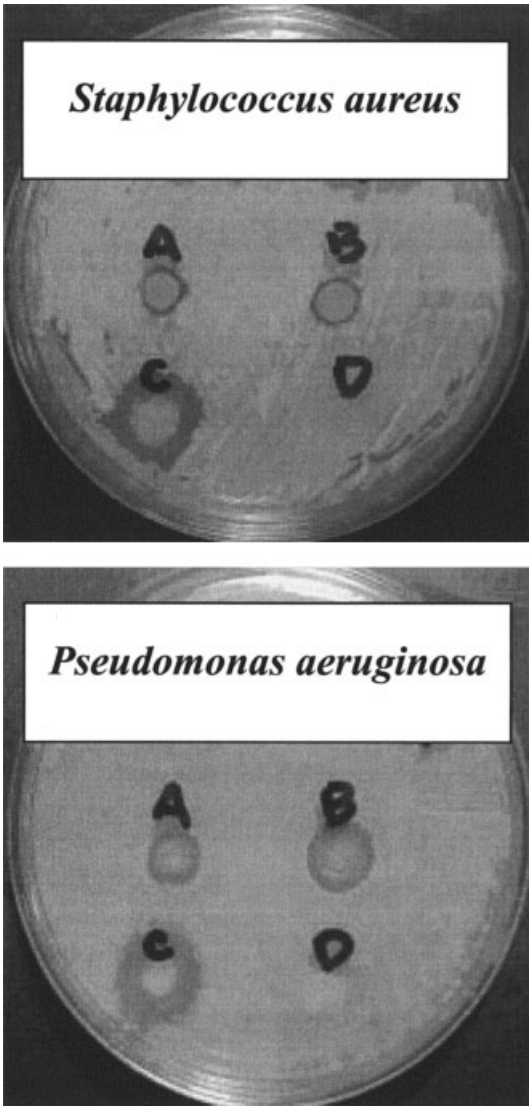

Figure 7 Photographic image of three types of PEC membranes and control group on culture plates inoculated with *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. Types of PEC membranes: (a) C1A7; (b) CA11; (c) CA15; (d) control.

benzoic acid (PABA) to folic acid to inhibit the growth of bacteria by analogues of essential metabolites. Because *P. aeruginosa* has a thinner cell wall than that of *S. aureus*, sulfadiazine easily penetrates into the cell wall of *P. aeruginosa* to interfere with its metabolic pathway. This is indicated by the results that the antimicrobial capability of AgSD-incorporated chitosan–alginate PEC membranes against *P. aeruginosa* was more effective than that against *S. aureus* [Fig. 9(a)].

It was also found that the chitosan–alginate interpolyelectrolyte complex indeed affects the release of AgSD. The CA15 membrane was most effective at inhibiting the growth of *S. aureus* and *P. aeruginosa* among the three types of PEC membranes. This can be attributed to the fact that the CA15 membrane has low stability, and the chitosan–alginate complex can be gradually broken down to release free alginate and chitosan under incubation in agar plates. Chitosan is a positively charged polyelectrolyte. It is recognized that the main component of the Gram-negative cell wall is lipopolysaccharide. Additionally, phospholipid, protein, lipoprotein, and a small amount of peptidoglycan are present, whereas the Gram-positive cell wall is composed of peptidoglycan as well as polysaccharide and teichoic acids. Its glycan strands of peptidoglycan are chains of alternating residues of *N*-acetylglucosamine and *N*-acetylmuramic acid, which are β-1,4-linked. The positively charged chitosan can form electrostatic attraction with the negative charged cell wall components of Gram-negative and -positive bacteria, such as lipopolysaccharide, phospholipid, teichoic acids, and *N*-acetylmuramic acid. The interaction between the positively charged chitosan and the negatively charged microbial cell wall leads to the leakage of intracellular constituents [Fig. 9(b)]. This

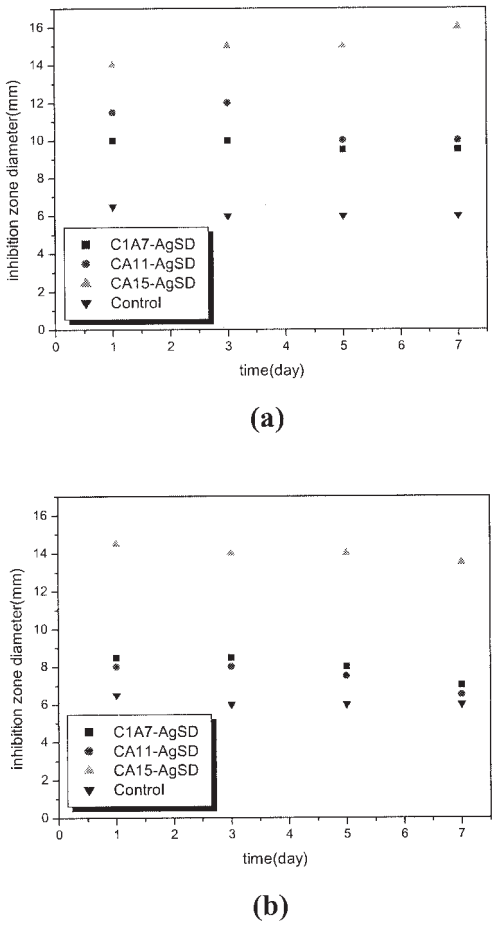

Figure 8 Inhibition zone versus culture time for various AgSD-incorporated chitosan–alginate PEC membranes. Species of bacteria inoculated in the agar plate: (a) *Pseudomonas aeruginosa*; (b) *Staphylococcus aureus.*

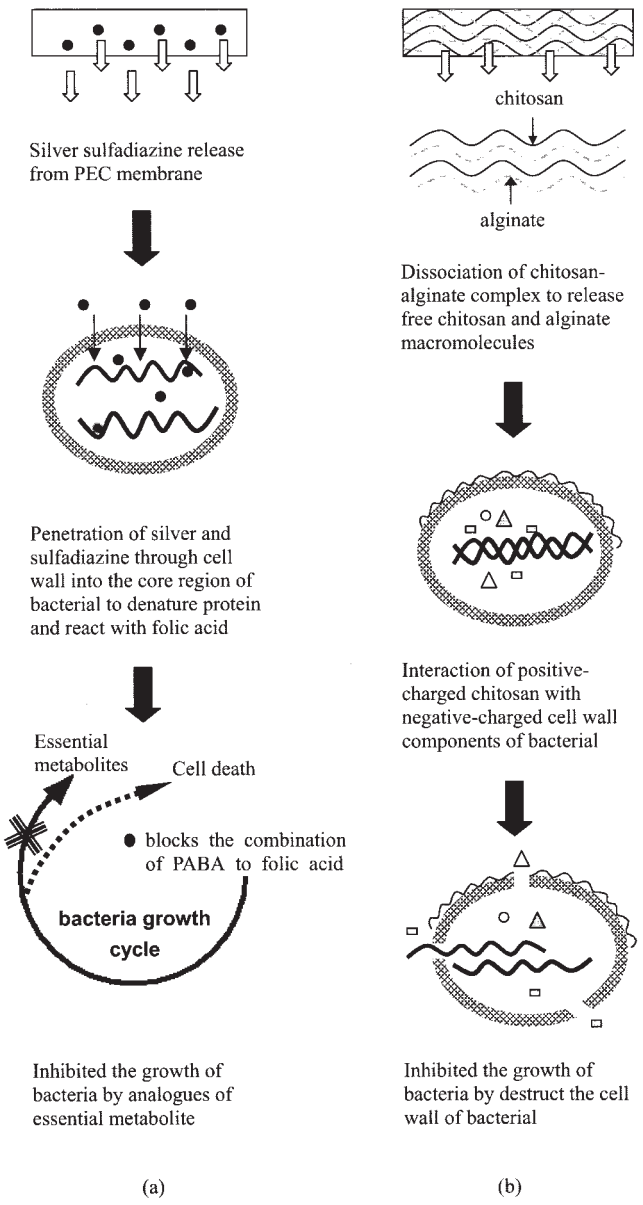

Figure 9 Inhibited bacterial cell growth pathway by chitosan–alginate PEC membrane incorporated with AgSD: (a) against bacterial cell by AgSD, (b) against bacterial cell by positively charged chitosan.

explains why the CA15 membrane has the most effective antimicrobial capability against *S. aureus* and *P. aeruginosa* among the three types of PEC membranes. The released chitosan from gradually disintegrated chitosan–alginate PEC and the released silver sulfadizane increase the antibacterial ability of the CA15 chitosan–alginate membrane.

CONCLUSIONS

The development of generalizable techniques for preparing chitosan–alginate polyelectrolyte complex in wound repairing continues to be of great interest. We have demonstrated that a homogenizing interpolyelectrolyte complex method can be used to prepare chitosan–alginate PEC membrane with different properties. Chitosan of low pH value appears to react more completely with alginate than chitosan of higher pH value, which results in the formation of a ladder-type of chitosan–alginate PEC membrane with high stability. In contrast, alginate of high pH value is suitable to react with chitosan for the preparation of a stable ladder-type of chitosan–alginate PEC membrane. Ionic crosslinking is helpful to increase the stability and reduce the solubility of chitosan–alginate PEC membrane in PBS solution. The profile of AgSD release and the culture of chitosan–alginate PEC membrane in agar plate against *P. aeruginosa* and *S. aureus* suggest that the PEC membrane containing antimicrobial agents can effectively suppress bacterial proliferation to protect wounds from bacterial invasion.

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