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Fabrication of asymmetric chitosan GTR membranes for the treatment of periodontal disease

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ABSTRACT

The asymmetric chitosan membranes were developed for the guided tissue regeneration (GTR) by using the two-step phase separation in this study. The bicontinuous structure on the top layer would be formed due to the liquid–liquid demixing by non-solvent induction and the pore size was ranged from 0.5 to 2 μm. The interconnected cellular pores ranged from 80 to 120 μm on the bottom layer would be created by the formation of ice crystals. The membrane developed in this research possessed good biocompatibility, tissue integration, cell occlusivity and osteoconduction, which would last for at least 3 months. The chitosan membrane also successfully prevented the proliferation of bacterial, which was significantly superior to all the commercial GTR products for now. The asymmetric structure would be appropriate for the release of complex drugs with different effective periods. The results showed that the asymmetric chitosan GTR membranes prepared in this study are promising for the treatment of periodontal diseases.

1. Introduction

Guided issue regeneration (GTR), a technique by using asymmetric membranes, is the most popular and efficient approach to treat serious periodontal disease nowadays (Gottlow, Nyman, Karring, & Lindhe, 1984; Hermann & Buser, 1996; Nyman, Lindhe, Karring, & Rylander, 1982). The biocompatible asymmetric membrane plays an important role in GTR, including the occlusion of cells from surrounding soft tissues and providing spaces for the remodeling of alveolar bones (Hermann & Buser, 1996; Maqut & Jerome, 1997). However, there are still some points needed to be improved for commercialized GTR membranes, including tissue integration, the suppressing of bacterial, the cell occlusivity and the transport of body fluids (Hermann & Buser, 1999; Mueller, Shortkroff, Schneider, Breinan, & Yannas, 1999).

In this research, chitosan, the biocompatible material with anti-biotic activity (Chio et al., 2001; Jia, Shen, & Xu, 2001; Sano, Shibasaki, Matsukubo, & Takaesu, 2003), was used to prepare GTR membranes, since the plaque of bacterials would be the main cause of the periodontal disease. In order to achieve the aims of tissue integration and osteoinduction, the micro-porous structure of chitosan GTR membrane would be necessary. On the other hand, the chitosan membranes have to prevent the invasion of surrounding soft tissues but allow the penetration of nutrients, water, air and wastes. It means that the asymmetric structure would be required for the GTR membrane (Hermann & Buser, 1996).

The freeze-gelation method (Ho et al., 2004; Hsieh, Hsieh, Liu, Wang, & Hou, 2006) was applied to prepare symmetric chitosan membrane. To fabricate the asymmetric GTR membrane, the freeze-gelation method would be modified as two-step phase separation in this study instead. The convectional liquid–liquid demixing process can also be used to prepare asymmetric membrane. However, the dense layer resulted from the liquid–liquid demixing lacks pores (Yanagishita, Nakane, & Yoshitome, 1994), which causes the barrier in mass transportation and tissue integration. On the contrary, the convectional freeze-gelation method can only create membranes with large pores (Ho et al., 2004) which cannot prevent the invasion of surrounding fibroblastic tissues. Thus, the freeze-gelation process was modified to overcome the above problems. Then, the experiments of anti-bacterial activity and *in vitro* cell culture would be carried out to verify the obstruction to gingival fibroblasts, anti-bacterial activity, tissue integration and osteoconductivity of this membrane.

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2. Materials and methods

2.1. Materials

Chitosan used in the present work was purchased from Sigma with a deacetyl degree of 85%. Acetic acid, sodium hydroxide (NaOH), ethanol, 3-(4,5-dimethythiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) and glutaraldehyde were all purchased from Sigma. All solvents are of analytical grade. For cell culture, α-minimum essential medium (αMEM), sodium β-glycerophosphate, ascorbic acid, and dexamethasone were purchased from Sigma, and fetal bovine serum (FBS), penicillin–streptomycin–amphotercin, trypsin–EDTA solution from GIBCO BRL.

2.2. Preparation and characterization of chitosan membranes

Chitosan powder was dissolved in an acetic acid aqueous solution (1 M) to form a 2 wt% polymer solution. The solution was used to prepare chitosan membranes. Porous chitosan membranes were prepared with the so-called freeze-gelation method, with details described in our previous work (Ho et al., 2004). However, in order to create asymmetric porous structure which is necessary for GTR membrane, the freeze-gelation method was modified in this research. Firstly, the 2 wt% chitosan solutions were placed in an aluminum dish and in contact with 1 M NaOH solution for different periods (2 min, 1 h and 3 days) at room temperature. In this step, the gelation occurred in chitosan solution from the interface coming into contact with NaOH solution. The chitosan solution with a gelled layer was then frozen at −20 °C, separated from the aluminum dish and immersed in a NaOH/ethanol aqueous solution (40 g NaOH powder in 1000 ml 50 vol% ethanol aqueous solution; the molarity of NaOH was 1 M and the pH value was 12.8) at −20 °C to allow the adjustment of pH and the gelation of chitosan. The gelled solution was dried at room temperature to remove the contained liquid. Asymmetric porous chitosan membranes were thus obtained.

For the comparison, the symmetric chitosan membrane was also prepared with the convectional freeze-gelation method. The detailed protocols were described in our previous work (Ho et al., 2004). Briefly speaking, the chitosan solution was frozen at −20 °C directly without the contact with NaOH solution at room temperature. Then, the frozen chitosan solution was separated from the aluminum dish and immersed in a NaOH/ethanol aqueous solution at −20 °C. Finally, the gelled solution was dried at room temperature to remove the contained liquid.

The size of chitosan membranes used for analysis and cell culture was 5 mm in length and width, and 2 mm in height. Morphologies of membranes were examined by using JEOL (JSM-6300) scanning electron microscope (SEM) after fixation, dehydration and critical point drying (CPD). The pre-treatment processes including fixation, dehydration and critical point drying were described previously (Ho et al., 2004).

2.3. Cells culture and analysis on chitosan membranes

Chitosan membranes were first fixed on culture dishes (Corning, USA) with silicone gel. ROS (rat osteosarcoma 17/2.8) or GF (human gingival fibroblast) cells suspended in the culture medium (5×10^4 cells/ml) were then added to the dishes to allow the ingrowth of cells onto the membranes which were 5 mm in length and width and 2 mm in height. The constituents in the medium were described as follows, and the medium was changed every 2 days. After incubation for various periods (1 and 2 weeks), cells on the membranes were harvested for analysis. For SEM analysis, membranes with cells were fixed with a 2 wt% glutaraldehyde solution and dehydrated in ethanol aqueous solutions. Drying with supercritical CO_2 was performed to prevent deformation of the cells attached to the membranes.

BCA protein assay is used to measure total protein concentration produced by the cells. The values obtained from BCA assay were applied in the ALPase analysis and cell inclusivity. In the former part, the chitosan membranes with cultured cells, maintained in culture for 1–4 weeks, were washed with PBS for three times to remove unattached cells. The washed membranes were then immersed in a trypsin–EDTA aqueous solution (0.05% w/v trypsin and 0.02% w/v EDTA) and incubated at 37 °C for 10 min to depart the attached cells. BCA protein assay was done by using Pierce BCA assay kit. After incubation, it could be quantified by using ELISA reader at wavelength of 562 nm. In the measurements, at least three replicates were used for each parameter.

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide) tests were respectively carried out at the cell culture of 3, 5 and 7 days, which was used to evaluate the cell activity. The MTT assay is a colorimetric assay for measuring the activity of enzymes that reduce MTT giving a purple color. These reductions take place only when reductase enzymes are active, which would be significantly related to the activity of mitochondria (Mosmann, 1983). In the beginning, the chitosan membranes with cultured cells were washed with PBS for three times, and 2 ml MTT solution (5 mg/ml) was added into the culture dishes for the reaction of 6 h at 37 °C. Then, the formazan dyes was dissolved by DMSO solution and quantified by using ELISA reader at a wavelength of 570 nm.

In the ALPase quantification assay, the fresh-prepared stock solutions used were 0.05 M sodium carbonate (as buffer), 2 mM *p*-nitrophenylphosphate and 2 mM magnesium chloride. The chitosan membranes with cultured cell were washed with PBS to remove unattached cells and immersed in lysis buffer for 10 min. Then, cell suspension solution of 0.4 ml was mixed with 1.2 ml stock solution and incubated at 37 °C. After 30 min, the reaction was stopped by using 2N NaOH for 30 min at 4 °C. Finally, ALPase was quantified by using ELISA reader at 405 nm. In the analysis of ALPase activity, the values from ALPase quantification assay are divided by the BCA value at the same time to get a specific ALPase activity.

In the BSA release experiment, the 10 wt% BSA solution containing anti-bacterials (PMSF 10 mM, sodium fluoride 50 mM and sodium azide 0.1%) was first prepared with sterilized PBS buffer. The chitosan GTR membrane was then immersed into the BSA solution for 24 h at 37 °C. After 24 h, the GTR membrane was re-immersed into a new PBS buffer where the loaded BSA was released. The PBS solution was analyzed after the releasing of 0, 0.25, 0.5, 1, 4, 7, 10 and 14 days for the concentration of BSA by using the BCA assay.

3. Results and discussion

3.1. The structure of GTR chitosan membrane

The structures prepared by convectional freeze-gelation process were shown in Fig. 1, and the detail process, mechanism, relative applications were referred to previous researches (Ho et al., 2004; Hsieh et al., 2006). Briefly speaking, in the convectional freeze-gelation process, the homogeneous polymer solution became heterogeneous after the freezing process because the decrease in solubility resulted from temperature changes. There would be two phases in heterogeneous solution, where one was the polymer-rich phase and the other was the polymer-poor phase. The transformation from one homogeneous phase into two phases was called "demixing". After the removal of solvent by using freeze-gelation process, the space originally occupied by the

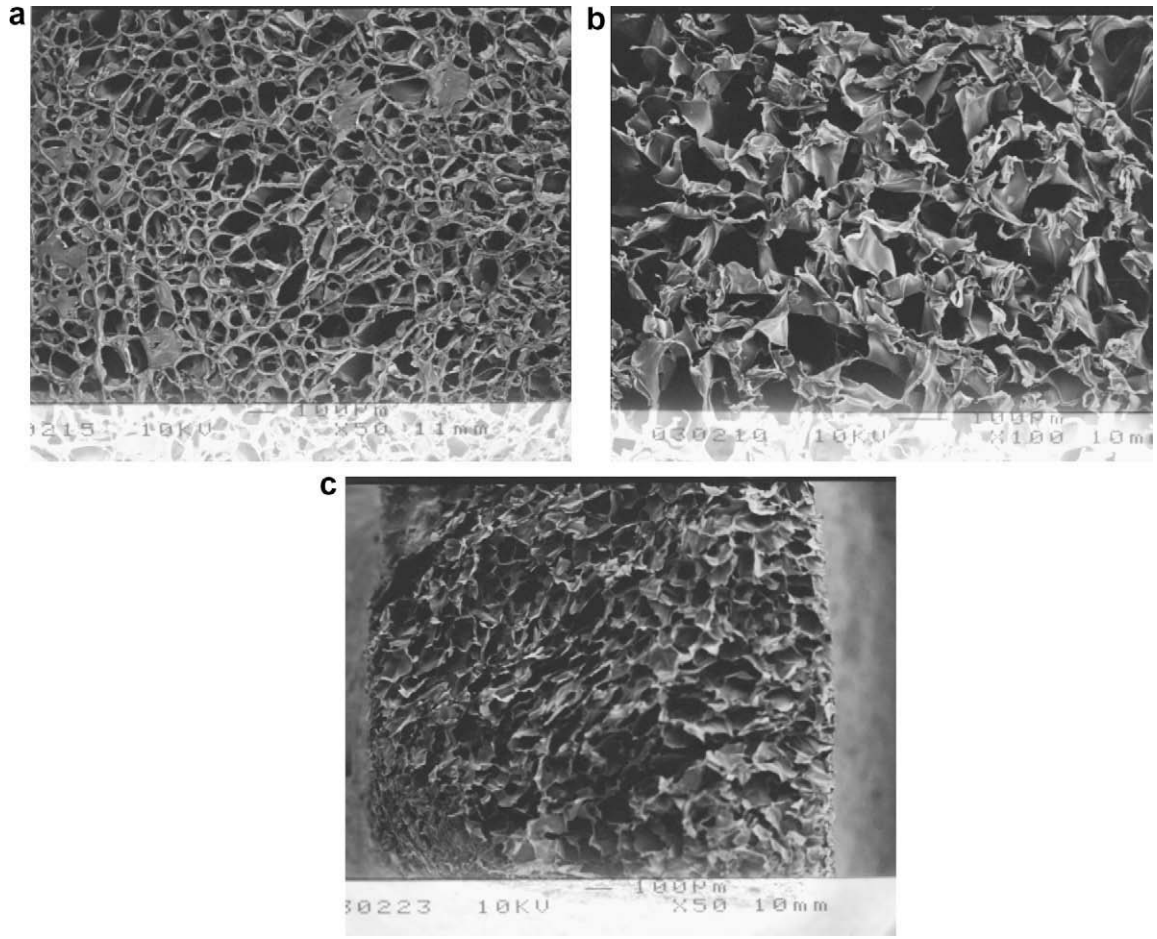

Fig. 1. The structure of symmetric chitosan membranes prepared with the convectional freeze-gelation method (a) top surface; (b) bottom surface; (c) cross section.

polymer-poor phase would become pores and the polymer-rich phase would becomes walls. Thus, the porous structures were obtained. By using convectional freeze-gelation process, symmetric porous membranes were prepared without skin layer, which would be beneficial for applications in the tissue engineering. However, the large pores in top layer cannot prevent the invasion from surrounding soft tissues although the same large pores in the bottom side would be good for osteoconduction as a GTR membrane. This is the reason why the freeze-gelation method was developed in this study to fabricate a membrane with asymmetric structure for GTR. The structure prepared by modified freeze-gelation method was shown in Fig. 2. By the comparison between Figs. 1 and 2, the top layer in Fig. 2 was obviously with smaller pores while the structure in Fig. 1 was symmetric and with large pores. This dense layer was just created by the novel step in the modified freeze-gelation method proposed in this research. The occlusion of cells was supposed to be reached by this dense top layer. The formation of this dense layer was described in the following paragraphs.

In the modified freeze-gelation process, the structures in chitosan membranes were developed in two stages in the preparation. On the top surface, the structures were formed in the contact with NaOH solution at room temperature, where the liquid–liquid phase separation would be dominant and resulted in bicontinuous and lacy-like pores. On the other hand, cellular pores on the other side were formed in the second stage where the temperature was lowered to −20 °C. In this step, the formation of ice crystals would produce large pores on the bottom side in the same chitosan membranes. For the membranes prepared with convectional freeze-gelation method, the chitosan solution was frozen at −20 °C directly, which was just the second stage in the modified freeze-gelation method, and there were only cellular large pores in the membrane. The asymmetric structures created by the modified freeze-gelation methods were indicated by SEM photographs in Fig. 2.

In the first step of modified freeze-gelation method, when the chitosan solution contacted with the alkaline coagulant solution (NaOH/water) at room temperature, the chitosan solution became turbid and the gelation occurred. The gelation was caused by the decrease in the solubility of chitosan, which induced the phase separation into polymer-rich and polymer-poor phases. The lacy structures with very small pores were produced by the demixing process in this step, which has been observed in the other polymer system (Ma & Zhang, 1999; Smolders, Van Aartsen, & Steenbergen, 1971). The pore size ranged from 0.5–1.5μm from SEM analysis (Fig. 2(a)). Because the polymer-rich and polymer-poor phases were all in the liquid state, this phase separation was also called "liquid–liquid demixing" (Smolders et al., 1971).

In the second step, the temperature was lowered to −20 °C. The temperature was much lower than the freezing point of the chitosan solution, so the solution was immediately frozen and solidified. The structures resulting from the freezing step were interconnected and cellular pores were ranged from 80 to 120 μm (Fig. 2(b)). The formation of ice crystals was the main reason for these macro-pores (Ho et al., 2004), which would be identified from the comparison with the picture in Fig. 2. Unlike the liquid–liquid demixing, the phase separation through the formation of ice crystals was very rapid, and the pores would be aligned if the freezing temperature was low enough.

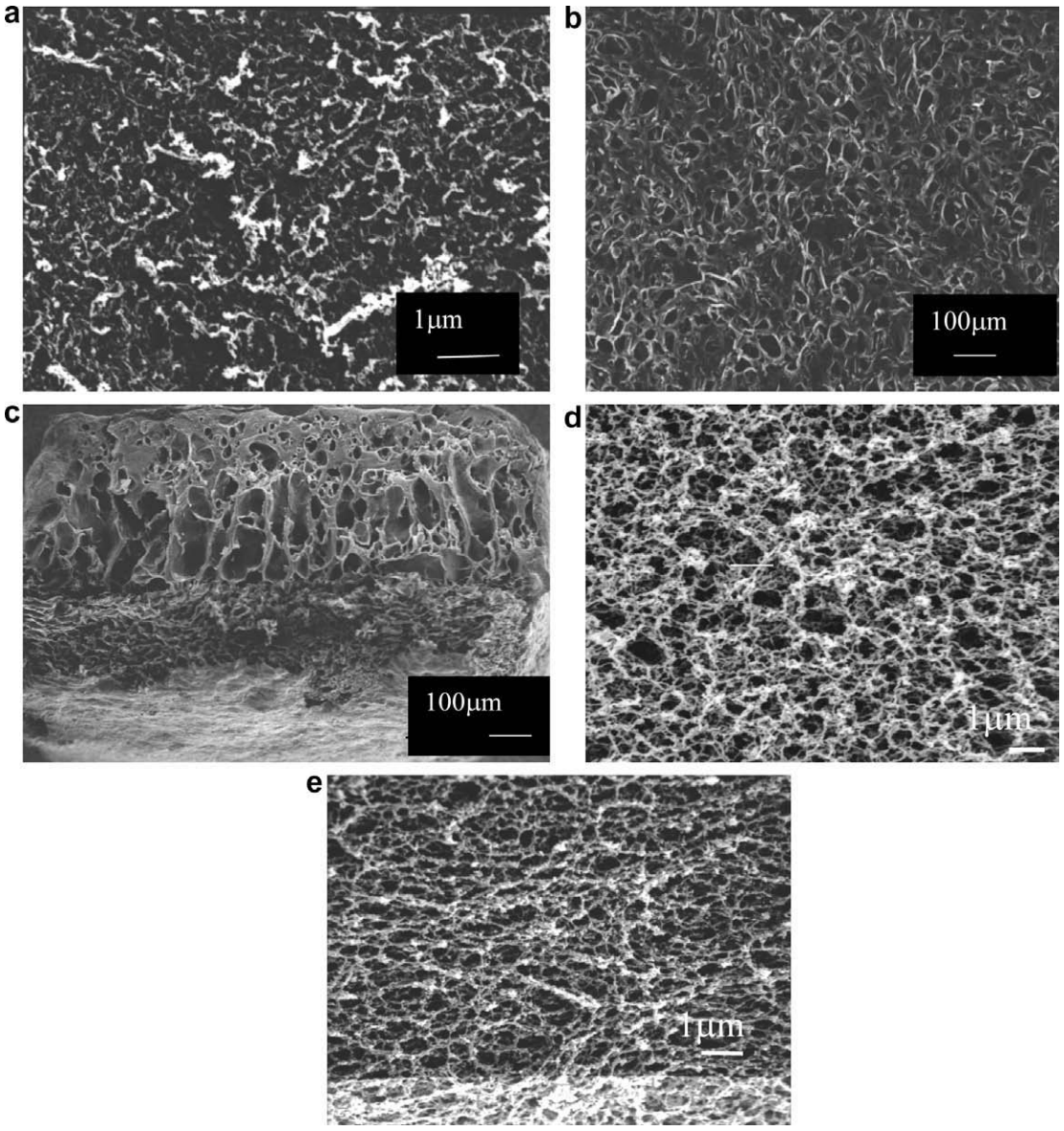

Fig. 2. The structure of asymmetric chitosan membranes prepared with the modified freeze-gelation method with the NaOH contact time at room temperature for 2 min (a–c) and for 3 days (d and e). (a) Top surface; (b) bottom surface; (c) cross section; (d) top surface; (e) bottom surface.

By the investigation of the interface between these two layers, the finger structures would be found as shown in Fig. 2(c). The walls of fingers were mainly composed of lacy structures, revealing that the structure was highly porous and interconnected. The existence of the finger structure proved that the top surface was formed due to the liquid–liquid demixing but not the formation of ice crystals. Smolder et al. (Berg & Smolder, 1992; Jacobs & Leukess, 1996; Ray, Krantz, & Sani, 1985; Young, Wang, Hsieh, & Chen, 1998) have pointed out that the instantaneous phase separation and liquid–liquid demixing would be two necessary conditions for the formation of finger structures in polymeric membranes. It therefore appears that the lacy structure was indeed caused by liquid–liquid demixing.

The thickness of macro-porous layer was determined by the penetration depth of alkaline solution in the first step. The membranes prepared with different immersion time were analyzed by SEM. With short immersion time (2 min) at room temperature, the bicontinuous layer would be thin as shown in Fig. 2(c). With long immersion time (1 h) at room temperature, the bicontinuous layer would be much thicker and the thickness of macro-porous layer decreased relatively. One of the extreme cases is shown in Fig. 2(d) and (e). To form the structures shown in these two figures, the chitosan solution was in contact with the basic coagulant for 3 days. There was no large and cellular pore on the bottom layer, and the bicontinuous structure thoroughly appeared in both the top surface (Fig. 2(d)) and the bottom surface (Fig. 2(e)). That was to say, the bicontinuous layer would grow with the time in contact with the NaOH solution at room temperature. The result also suggested that the bicontinuous structure with small pores was formed due to the NaOH solution. If the contact time was long enough, such as the groups shown in Fig. 2(d) and (e), the NaOH solution would penetrate through the membrane. Consequently, the structure resulting from liquid–liquid demixing occupied the whole membrane. In Fig. 2(d) and (e), there was not any large and cellular pore observed on the bottom side, suggesting that there was no formation of ice crystals in this system. This finding indicated that the formation of the ice crystals would be retarded if the liquid–liquid demixing first took place.

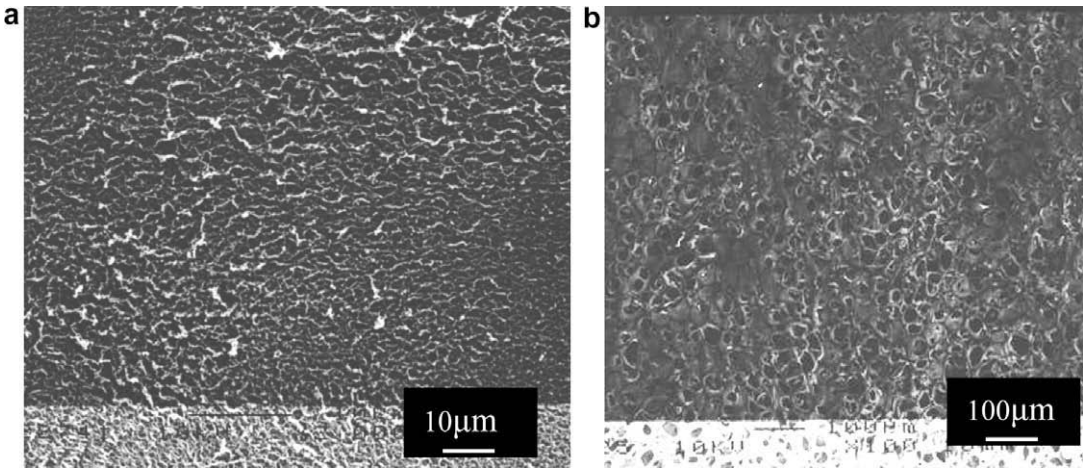

Fig. 3. The structures of chitosan membrane after the immersion in PBS for 91 days. (a) The top sides of chitosan membranes and (b) represents the bottom sides of chitosan membranes.

The above outcomes revealed that the penetration of NaOH in the first step took time. Only when the immersion time in the first step was long enough for the penetration of basic coagulant was a symmetric lacy-like structure obtained. On the other hand, after the immersion in NaOH with limited time, the polymer solution would be divided into upper and lower regions: the upper region fixed by liquid–liquid demixing and the lower region kept as a homogeneous solution without demixing. For the region with the liquid–liquid demixing, the polymer was mainly distributed in the polymer-rich phase. In liquid–liquid demixing, the chitosan concentration would be much higher in the polymer-rich phase, compared with the concentration of homogeneous solution before demixing. The high polymer concentration would increase the entanglement between chitosan chains, which has also been observed for the other polymers. With high entanglement degree, the movement of polymer chains would be suppressed, so ice crystals would not grow further. Thus, the ice crystals were only formed in the region without liquid–liquid demixinig. That was to say, the dominant demixing mechanism for structure formation would be liquid–liquid demixing for the region closed to the interface between NaOH and chitosan solution. For the homogeneous region where NaOH has not yet penetrated, the mobility of surrounding polymers was comparatively high. So, the ice crystals would grow immediately as the temperature was lowered in the second step. Obviously, the liquid–liquid demixing was the critical cause for the mobility of polymer chains in this research, which was important to determine if ice crystals would form in the freezing process. As a result, no significant ice crystals would be formed if the time for liquid–liquid demixing was long enough to restrain the polymer chains, where only the bicontinuous structure occurred. From the above analysis, we can control the thickness of the bicontinuous and macro-porous layers by adjusting the immersion time in the first step.

3.2. The stability of GTR chitosan membrane

The prerequisites for GTR membranes would include cell occlusivity, space making and tissue integration. The above properties are all highly related to the structure of the GTR membranes. Only with the appropriate structure can the chitosan membrane prevent the invasion of cells from surrounding tissues and maintain the necessary mass transfer through this membrane. In order to observe any possible difference in porous structure of the chitosan membrane, SEM was used to see if there was any structure collapse. In this part, the stability of GTR membranes is evaluated. That is, the chitosan GTR membrane is immersed into PBS buffer at 37 °C for 4 months. In this period, one membrane is analyzed by using SEM every 7 days. The results in Fig. 3 revealed that the structures of GTR membranes are maintained without obvious erosion in 6 months. Compared with the degradation of chitosan substrates in previous paper (Baran, Mano, & Reis, 2004), the chitosan erosion in this study would be slower. It is because the chitosan used in this research possesses high molecular weight, which makes polymeric degradation slower. This outcome suggested that the GTR membrane would be maintained longer than 4 months, which is ideal for applications in the regeneration of alveolar bones.

3.3. The anti-biotic and cell occlusivity effects of GTR chitosan membrane

In order to evaluate the potential of this asymmetric chitosan membrane for GTR treatments, anti-biotic analysis was carried out in this study. Before the measurement of anti-biotic activity, chitosan membranes were immersed in the PBS buffer for different periods from 1 week to 4 months which was just the implantation time in clinical use. The chitosan membrane after immersion was placed on the cultured dish with confluent *Escherichia coli*, and then the chitosan membrane was removed from the dish. From Fig. 4, the anti-biotic regions caused by the chitosan membrane were significantly clear in all the experimental groups. The results indicated the cultured bacteria were successfully suppressed when covered with chitosan membranes even when the chitosan membrane has been degraded for 4 months. That is to say, with the chitosan GTR membranes, the plaque which was the major cause of periodontal diseases would be highly suppressed. By the easing of bacteria infection, the treatment of periodontal disease would be more efficient.

For the evaluation of cell occlusivity, GF cells were cultured on the side of small pores in asymmetric chitosan membranes and the scheme of culture system was described in Fig. 5(a). The protein deposition on the bottom of the culture dish would be examined and compared with the control group containing the medium only but without GF cells. The results of protein deposition are shown in Fig. 5(b). After culturing for 4 months, there was no difference in the protein deposition of the experimental and control groups. The finding suggested there was no GF cell intruding into the membrane, indicating that the asymmetric chitosan membrane prevents the invasion of cells from soft tissues for at least 4 months. In addition, the maintenance of cell occlusivity and anti-biotic activity on chitosan membrane also agree with the

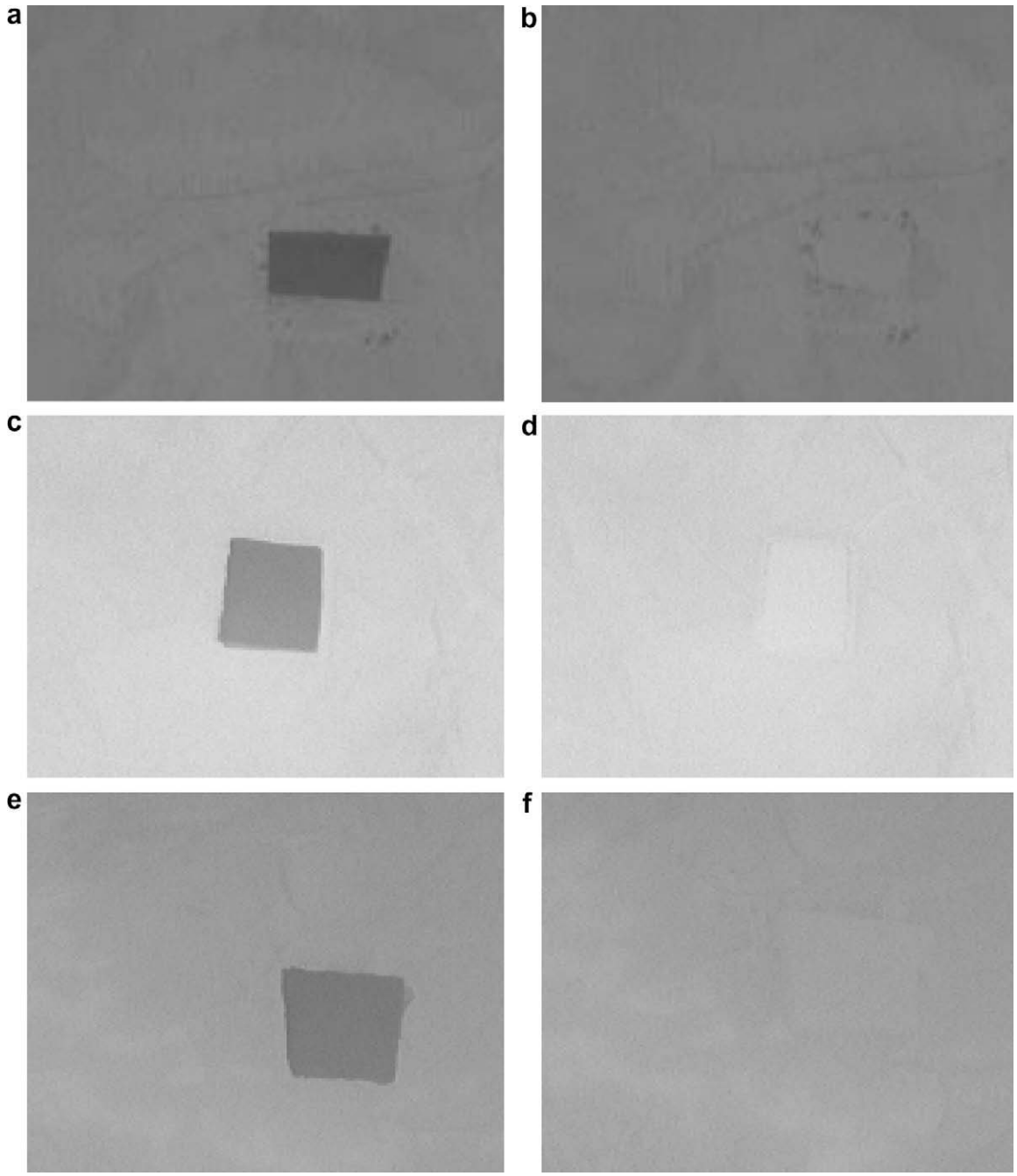

Fig. 4. The anti-biotic activities of asymmetric chitosan membranes after the immersion in PBS of (a and b) 14 days, (c and d) 56 days and (e and f) 126 days. (a, c and e) were the culture dish with *E. coli* and covered with chitosan membranes (as the dark area). (b, d and e) were pictures after the removal of chitosan membrane in (a, c and e), respectively.

results in Section 3.2. The degradation or erosion of chitosan membrane was not significant at all in 4 months, so the physical and chemical properties of membrane would not vary to any major extent. It appears that the stability of this GTR membrane was excellent.

To investigate the tissue integration and space-making effects, the osteoblastic cells, ROS, were cultured on the side of large pores in asymmetric membranes. For the cultured ROS cells on asymmetric chitosan membranes, the cells would attach well, spread well, and proliferate into the macro-pores of the chitosan membrane as shown in Fig. 6. From the above outcomes, the superiority in cell occlusivity and cell integration of the asymmetric chitosan membrane developed in this research is clear.

3.4. The cell proliferation and phenotypes in GTR chitosan membrane

For the MTT analysis, ROS cells were cultured on asymmetric chitosan membranes (macro-porous side), cellulose acetate membrane with pores of 0.22 μm, chitosan membrane with dense skin and commercialized culture dish. From Fig. 7, the cellular mitochondrial activity on asymmetric membranes was roughly the same with that on the commercial culture dish. However, the cell activities were extremely low when cultured on the membrane with a dense skin and very small pores (0.22 μm). The possible reason would be the decrease in mass transport caused by the existence of the very dense layer. The result demonstrated that the layer of lacy structures would not retard the transport of medium

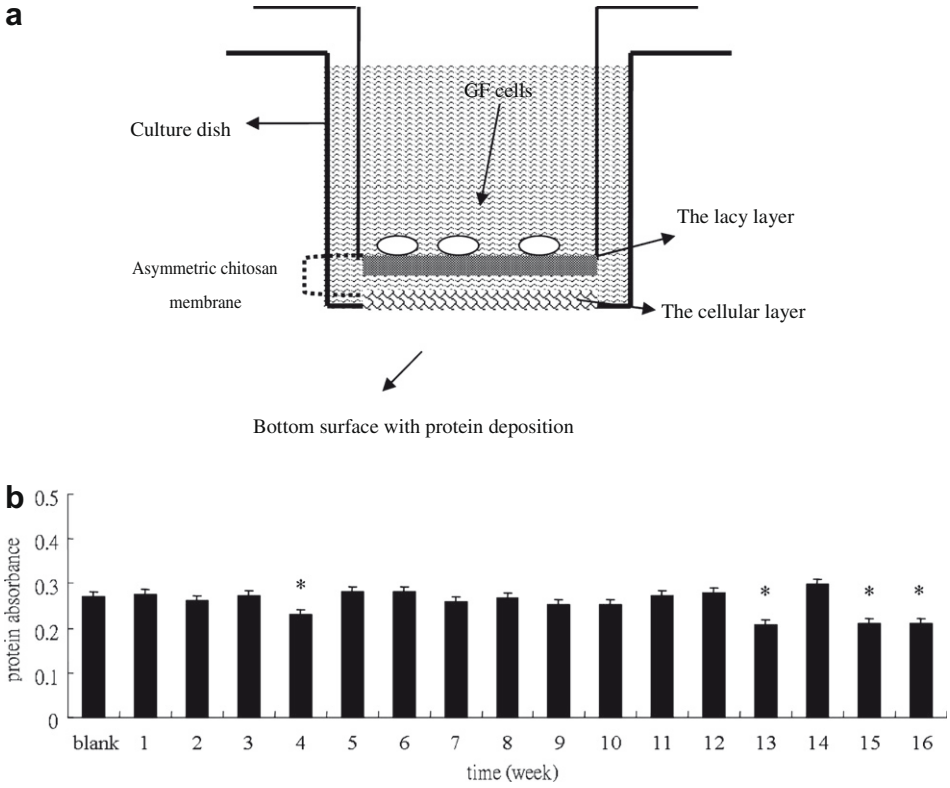

Fig. 5. The examination of cell inclusivity for the asymmetric chitosan membranes. (a) The scheme of experimental devices; (b) the results of protein deposition. In (b), the blank was carried without cell seeding, and the significance (by *t*-test) was indicated as $p < 0.05$ (*) compared with the value of blank.

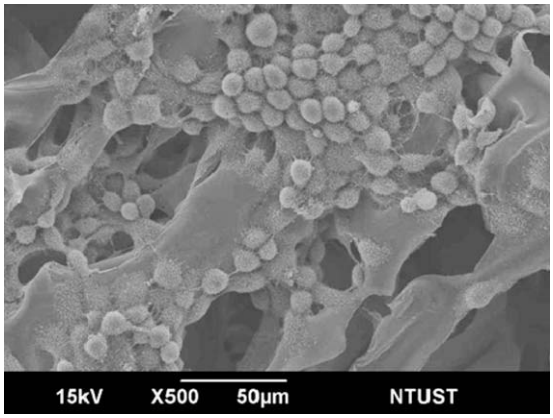

Fig. 6. The growth of ROS alls on the macro-porous side of asymmetric chitosan membranes.

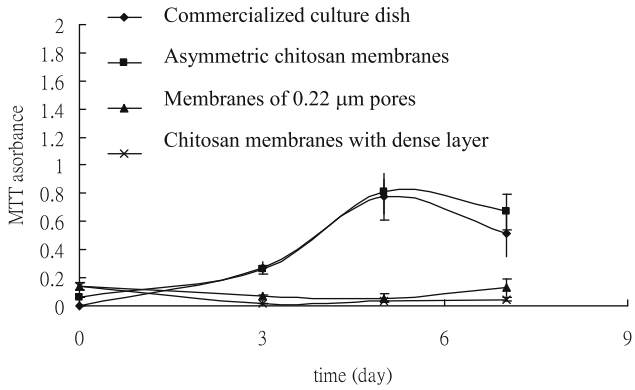

Fig. 7. The MTT analysis for osteoblastic cells cultured on commercialized culture dish, asymmetric chitosan membrane developed in this research, cellulose acetate membrane with the pore size of 0.22 μm and chitosan membrane with dense skin layer.

or body fluid, although it can efficiently prevent the invasion of gingival fibroblasts.

ALPase assay was carried out to realize of the activity of bone differentiation for ROS cells cultured with asymmetry chitosan membrane; besides, the commercialized PLA (poly(L-lactic acid)) GTR membrane was applied as the control group. The results shown in Fig. 8 indicated that the cells cultured with asymmetric chitosan membranes significantly expressed higher ALPase activity compared with cells cultured with PLA membranes. The finding revealed that the osteoconductivity of asymmetric chitosan membranes was clearly higher than commercialized GTR membranes.

Except for the maximum value of ALPase activity the expression time of ALPase also indicated that the better osteoinduction effect of chitosan membrane. In a general mineralization process, the ALPase activity would increase in the early osteoblastic differentiation, and then decrease in the further differentiation (Stein et al., 1996). That was to say, the cells on the chitosan membrane became further mineralized, so the ALPase started to decrease after the maximum peak appeared. On the contrary, the expressed ALPase on PLA membrane was kept an almost constant value in the culture period. The result indicated that the cells on this commercial membrane have not entered the further differentiation even after culturing for 17 days.

3.5. The controlled release by GTR chitosan membrane

For the treatment of periodontal diseases, the GTR membrane would possess higher potential if some drugs, such as growth

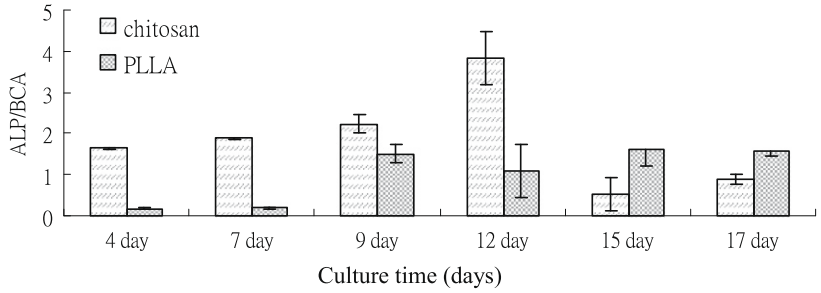

Fig. 8. The ALPase activity of osteoblastic cells cultured on asymmetric chitosan membrane developed in this research and commercialized PLA membrane for GTR.

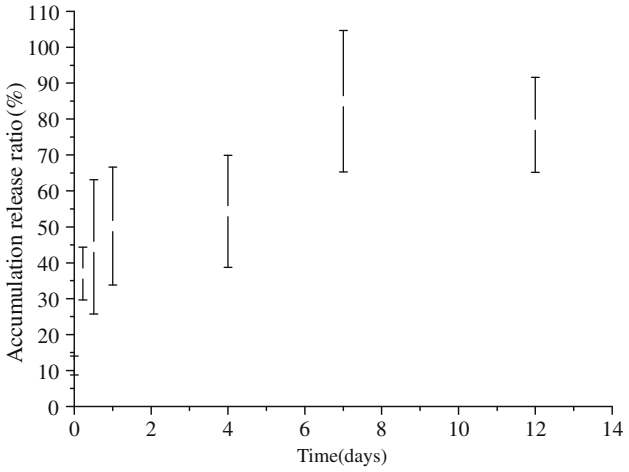

Fig. 9. The releasing profile of BSA from chitosan GTR membranes under 37 °C.

factors, anti-biotics or the others, can be released continuously and effectively. In this research, the protein, BSA (bovine serum albumin) was loaded into the GTR chitosan membrane to evaluate the ability of controlled release by using this novel GTR membrane. The results are described in Fig. 9. Fig. 9 indicated that the release of protein would be divided into two stages. In the beginning (0–4 days), the release is faster and the accumulation releasing amount would reach 50%. Between 4 and 12 days, the accumulation release of proteins is slower and would increase gradually to 80%. The two-stage release is possibly due to the asymmetric structure in chitosan GTR membranes. In this research, the pore size was ranged from 80 to 120 μm in the bottom side. According to the results in the previous research (Hsieh et al., 2006), the proteins would be mostly released in 4 days if the porous structure was similar to macro-pores on the bottom surface in this study. On the other hand, the release period by using lacy-structured chitosan membrane would be ranged from 4 to 10 days (Mi, Wu, Shyu, & Chao, 2003), which roughly agreed with the results in this research. Thus, it is reasonable to infer that the protein release would be divided into two parts in the asymmetric GTR membrane developed in this research. For the proteins carried by the bottom sides with large pores, the release rate would be higher due to the lower resistance in mass transportation. Comparatively, the resistance of protein release in the top layer would be higher because the structures provided higher resistance. Based on the above results, it is proved that there would be a two-stage release by using the asymmetric chitosan GTR membrane prepared in this study, including a short-term release in 4 days and a long-term release in 10 days. Therefore, the GTR membrane developed in this research can be practically applied in the controlled release of complex drugs with different releasing profile at the same time. That is to say, the multi-functional treatment can be reached by releasing various drugs or biomolecules to promote the regeneration of periodontal tissues more effectively.

4. Conclusion

In this study, novel biocompatible GTR membranes with asymmetric structures have been successfully prepared with chitosan by the two-step phase separation process which was modified from the freeze-gelation method. The top surface of the membrane effectively prevented the invasion of human GF cells; on the other hand, the bottom surface provided enough space for the growth and regeneration of bone tissues. Besides, the strong anti-bacteria activity would promote the effect of GTR membrane on the treatment of periodontal disease. The osteoblastic cells cultured with the asymmetric chitosan membrane also expressed higher cellular activity and more significant osteoblastic phenotypes, compared with the cells cultured on commercial GTR membranes of PLLA. In the drug release experiment, the novel GTR membrane was proven to be proper for the multi-staged delivery. The results proved that the potential of this novel GTR membrane in the treatment of periodontal disease was excellent.

Acknowledgments

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