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Preparation of alginate membrane for tissue engineering

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Abstract: Sodium alginate was provided with good processibility according to physical and chemical characterization of itself. Alginate scaffold has been used for preparation of soft or hard tissue engineering, but the structure of the scaffold needs to be improved for better performance for skin tissue engineering. In this study, highly porous alginate membrane was formed with ionic crosslinking. High molecular weight (M_w=3.0×10^5) alginate showed the best film-forming property. Therefore, the appropriate molecular weight should be selected for improving its performance. With freeze-drying technology and pre-freezing at -10°C, we have built the honeycomb materials (porosity=92.06%). Changing the pre-freezing temperature can regulate pore structure to some extent. With the increased dosage of sodium alginate, the porosity and the pore size of the materials were reduced, whereas tensile strength and elongation at break increased. Water absorption performance of the materials was good. The above studies lay a foundation for construction of skin tissue engineering scaffold.

Keywords: alginate; freeze drying; membrane; tissue engineering.

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1 Introduction

Scaffolds for tissue engineering and drug release control systems require materials with an open porous structure, which can enhance absorption and adsorbent performance, provide space for mass exchange, and facilitate the apposition growth of cell tissues [1]. The new material system developed in the 20th century uses natural polymer materials with porous structures that are widely used in different fields, especially in tissue engineering [2–3].

Sodium alginate is a kind natural polymer for tissue engineering, which consists of different lengths of G (a-L-Gluronie), M (β-D-mannuronie), and GM alternating blocks [4]. The formula of sodium alginate is $(C_6H_7O_6Na)_n$, and the theoretical value of the molecular weight of sodium alginate is 198.11. Sodium alginate can react with divalent or higher metal ions [5]. In 1944, Speakman and Chamberlain [6] first used a variety of metal ions for the ion exchange with sodium alginate, which resulted in different kinds of alginate fibers, such as calcium alginate, ferrum alginate, and aluminum alginate.

Sodium alginate with different molecular weights is used in the medical field [7]. Changing the molecular mass of sodium alginate can alter the mechanical properties of ionic crosslinked alginate calcium scaffold. However, changing the molecular mass cannot degrade the enzymes of sodium alginate [8]. Sodium alginate has a high molecular weight. Therefore, the direct excretion of sodium alginate is difficult. The difficulty in sodium alginate excretion limits its application to a particular extent. However, sodium alginate with a low molecular weight (M_w<4.8×10^4) can be excreted by the body's metabolism [9]. Sodium alginate with a low molecular weight can accelerate the growth of keratinocytes, improve the migration rate of endothelial cells, and promote the proliferation of fibroblasts. However, the mechanical properties of hydrogel or porous membranes created from sodium alginate with an excessively low molecular weight cannot meet the basic requirements of medical dressings or scaffolds for tissue engineering [10]. Therefore, the application range of molecular weight should be clarified.

Freeze-casting technology has been used for preparing tissue engineering scaffolds, for example, polyelectrolyte complex fibers consisting of chitosan and sodium hyaluronate were prepared with this technology, which were the natural oppositely charged biopolymers [11, 12].

In search of suitable molecular weights of alginate for scaffolds with excellent properties, our study used sodium alginate with different molecular weights to prepare alginate membrane via freeze-casting. The chemical structure, pore size, porosity, water absorption rate, moisture content, and tensile property of alginate membranes prepared at different pre-freezing temperatures and concentrations were analyzed and characterized.

2 Materials and methods

2.1 Materials

Sodium alginate of three different specifications (M_w=3.0×10^5, M_w/M_n=1.5; M_w=1.48×10^5, M_w/M_n=3.0; M_w=2.8×10^4, M_w/M_n=1.4, G/M as 2/1, medical level, Qingdao Hyzlin Biology Development Co., Ltd., Qingdao, Shandong, PRC) and calcium chloride (analytically pure, Sinopharm Chemical Reagent Co., Ltd., Beijing, PRC).

2.2 Preparation of alginate membrane

Sodium alginate solutions were divided into three groups according to different concentration (1.5%, 2.5% and 3.5%). They were dissolved in water by stirring and processed via deaeration in 12 h at 4°C. The each group was pre-frozen at different temperatures (-10°C, -20°C, and -60°C) for 12 h and processed in a vacuum freeze dryer (LGJ-10, Beijing Songyuan Huaxing Technology Co., Ltd, Beijing, PRC). Next, 5% calcium chloride was used for crosslinking. Subsequently, the specimens were pre-frozen for another 12 h and processed via freeze-casting technology to create alginate membrane.

2.3 Fourier transform infrared spectroscopy characterization before and after sodium alginate crosslinking

Sodium alginate was made into a solution with a mass percent of 0.25%. The solution was dried and made into a sodium alginate membrane. The sodium alginate prepared via calcium chloride crosslinking was also dried into a thin membrane. The membranes were tested using an infrared spectrometer (Nicolet-5700, Thermo Electron Co., Ltd., MA, USA). Changes in the sodium alginate group were characterized.

2.4 Morphologic observation and pore size measurement of alginate membrane

The macro-morphology of alginate membrane was observed and recorded using digital cameras. In addition, the surface and a section of alginate membranes coated via ion sputtering was placed under a scanning electron microscope (JSM-6390LV, JEOL Ltd., Akishima, Tokyo, Japan) to observe the pore structure, microstructure, and connection degree of alginate membranes. The average pore size was also measured by Image-Pro Plus (Media Cybernetics, Inc., Rockville, MD, USA).

2.5 Porosity characterization of alginate membrane

In this study, the scaffold porosity was measured via the liquid displacement method. The specimens were cut into pieces with a size of 1 cm×1 cm. The cut specimens were combined with absolute ethyl alcohol (analytically pure, Sinopharm Chemical Reagent Co., Ltd., Beijing, PRC) with the volume of V_1. After 10 min, the ethyl alcohol had sufficiently entered the pores of the porous scaffold until no more bubbles were produced. At this moment, the volume of ethyl alcohol (immersing the scaffold) was recorded as V_2. Scaffold specimens immersed in ethyl alcohol were taken out, and the volume of the remaining ethyl alcohol was recorded as V_3. The scaffold porosity (P) can be calculated using Eq. (1) [13]:

$$P=\frac{(V_1-V_3)}{(V_2-V_3)}\times 100\% \quad (1)$$

2.6 Water absorbency test of alginate membrane

An alginate membrane with an area of 1 cm×1 cm was cut. The dried weight of the specimen was taken as m (g). The dried specimen was immersed in deionized water for 24 h and then removed. The surface moisture was quickly absorbed using filter paper, and the wet weight was taken

as m_1 (g). Water absorbency W_m can be calculated using Eq. (2) [14]:

$$W_m=[(m_1\text{-}m)/m]\times 100\% \quad (2)$$

2.7 Tensile property characterization of alginate membrane

Our study used the fabric thickness gauge to measure the thickness of the specimen. We also used the universal material testing machine (5500R, Instron, MA, USA) to test the mechanical properties of the alginate membranes. The clip distance was 50 mm, and the rate of extension was 5 mm/min.

The tensile strength (σ_t, MPa) and the breaking elongation (ε_t, %) of the membranes were calculated using Eqs. (3) and (4) [15]:

$$\sigma_t=p/(b\times d) \quad (3)$$

where p is the tension at specimen rupture, N; b is the specimen width, mm; and d is the specimen thickness, mm:

$$\varepsilon_t=(\Delta L/L)\times 100\% \quad (4)$$

where L is the initial distance between fixtures, mm; and ΔL is the distance increment between fixtures, mm.

3 Results

3.1 The effect of molecular weight on the membrane-forming property of alginate membrane

In sodium alginate, the C-O vibration of -COO- was weak and no absorption peak appeared (see Figure 1). The stretching vibration peak of C-O of the alginate membrane that appeared at 1266 cm^{-1} was attributed to the reaction between carboxyl and Ca ion ("C-O-Ca-O-CO-" group structure), which enhanced C-O vibration [16]. These changes indicate that Ca ion formed an "egg tray" structure with the sodium alginate molecular chain.

The pre-freezing temperature used in this study was -10°C, and the concentrations of sodium alginate were 1.5%, 2.5%, and 3.5%. The alginate membrane was white and the alginate membrane with $M_w=3.0\times10^5$ was the softest (see Figure 2). All three concentrations of sodium alginate were able to form a membrane. The alginate membrane with $M_w=1.5\times10^5$ also formed a membrane, but cracks in the membrane were seen (see Figure 3). The

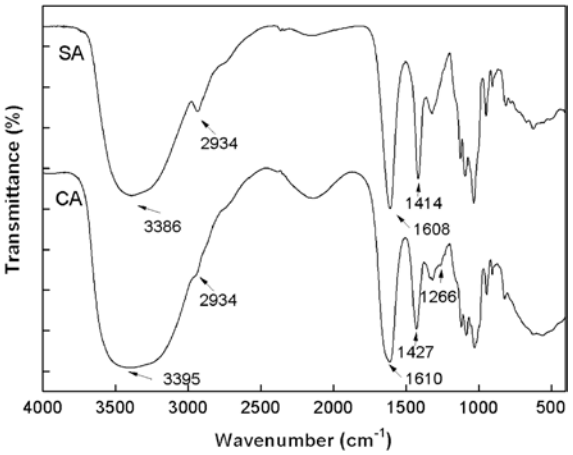

Figure 1: Fourier transform infrared (FTIR) spectra for sodium alginate (SA) and calcium alginate (CA).

alginate membrane with $M_w=2.8\times10^4$ was not conducive for membrane formation (see Figure 4). All three dosages of sodium alginate showed comparatively large cracks, and the membrane was difficult to form. The results indicate that the molecular weight was significantly influenced by the membrane-forming property of the alginate membrane. A large molecular weight indicates a high membrane-forming property [17].

3.2 Influencing patterns of pre-freezing temperature on the structure of alginate membrane

In this study, the alginates ($M_w=3.0\times10^5$, 1.5%) were selected, and different temperatures (-10°C, -20°C, and -60°C) were selected to explore the influence of pre-freezing temperature on the structure of the alginate membrane (see Table 1 and Figure 5). At -10°C, most of the pores on the surface of the alginate membrane had pore diameters varying from 150 μm to 200 μm. The average pore size of the alginate membrane was 234.50 μm, which was bigger than at the other temperatures (-20°C and -60°C). The formation rate of crystal nucleus in the nucleation was relatively slow, but the growth rate of crystals was rapid. The volume of the crystal was relatively large, and the pore diameter after the sublimation was also large.

At the crystal growth stage and at a high pre-freezing temperature, the temperature gradient of the solution was not significant, and the crystal almost had a hexagonally symmetrical shape. The crystal grew along the six main axes. Several sub-axes were extended and connected with other crystals and established a network structure in the sodium alginate solution. After freezing and drying,

Figure 2: Photographs of alginate membranes with high molecular weight. (A) 1.5%; (B) 2.5%; (C) 3.5%.

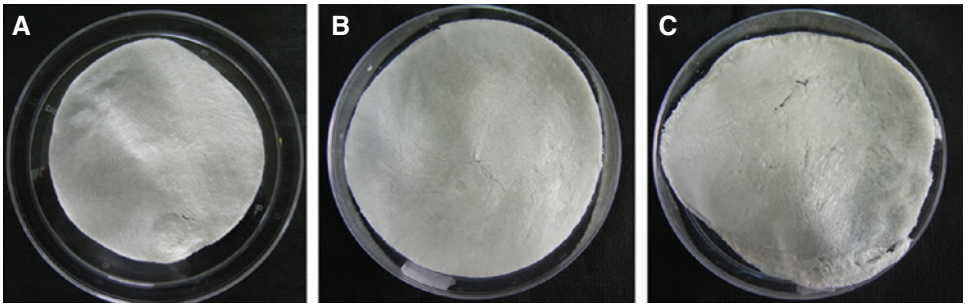

Figure 3: Photographs of alginate membranes with medium molecular weight. (A) 1.5%; (B) 2.5%; (C) 3.5%.

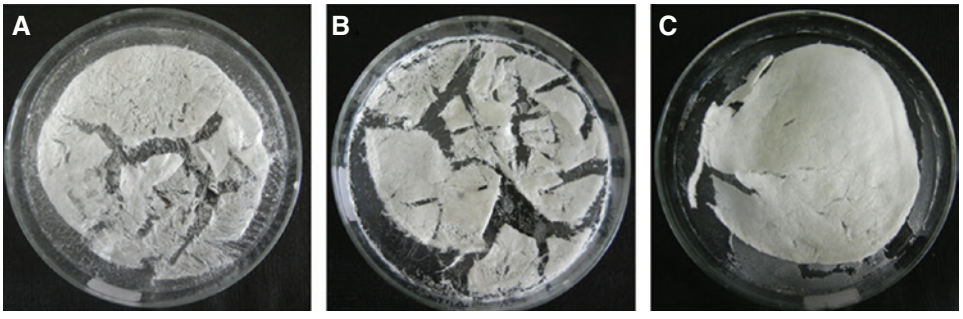

Figure 4: Photographs of alginate membranes with low molecular weight. (A) 1.5%; (B) 2.5%; (C) 3.5%.

Table 1: Average pore size and porosity of alginate membrane at different pre-freezing temperature.

Pre-freezing temperature (°C)	-10	-20	-60
Average pore size (μm)	234.50±78.24	208.10±38.21	157.70±79.53

cellular pores were left and appeared like a sponge. The surface of the sodium alginate solution was in contact with air. The surface tension of the sodium alginate solution was relatively large because of the air molecules. To reduce the tension, sodium alginate molecules in the solution had to move toward the solution surface. Therefore, the surface concentration of the sodium alginate solution increased. The pore diameter of the upper surface was relatively small. In general, when the pre-freezing temperature was decreased, the pore diameter decreased [18]. The surface pore diameter was smaller than the pore diameter of the cross section.

3.3 Effects of sodium alginate dosage on the structure and performance of alginate membrane

The pre-freezing temperature used in this study was -10°C, and the pores of the alginate membrane were cellular-shaped (see Figure 6). When the sodium alginate content

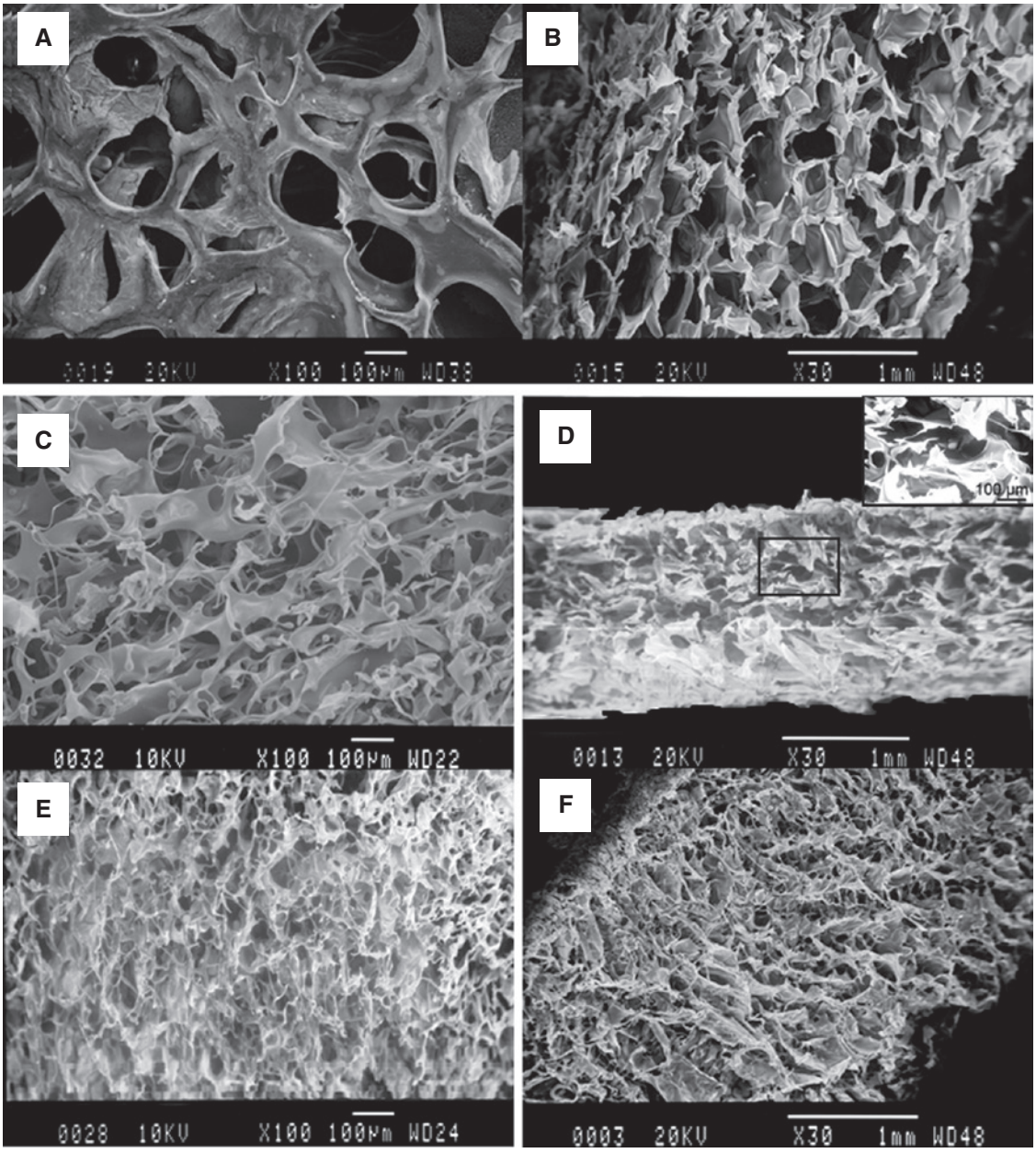

Figure 5: Surface (A, C and E) and cross section (B, D and F) scanning electron microscopy (SEM) images of alginate membrane at different pre-freezing temperatures. (A, B) -10°C, (C, D) -20°C, (E, F) -60°C.

was increased in the solution, the pore diameter of alginate membrane decreased. The decrease in pore diameter was attributed to the enlarged molar ratio of sodium alginate in unit volume. Meanwhile, the acting force of hydrogen bonds between molecules was enhanced, which decreased the diameter of the pores. More importantly, the content of sodium alginate directly influenced the viscosity of the solution. When the content of sodium alginate was increased, the viscosity of the solution was enhanced. The transmission of water and sodium alginate molecules was hindered, which resulted in a decrease in crystal size. Therefore, the pore diameter was negatively correlated with the sodium alginate content.

In the presence of sodium alginate, the water absorbency of the alginate membrane in deionized water initially increased and subsequently decreased (see Figure 7). The water absorbency of the alginate membrane was higher than 1000%, which was much higher than the water absorbency of the sodium alginate hydrogel without pores (157.38%). The water absorbency of the

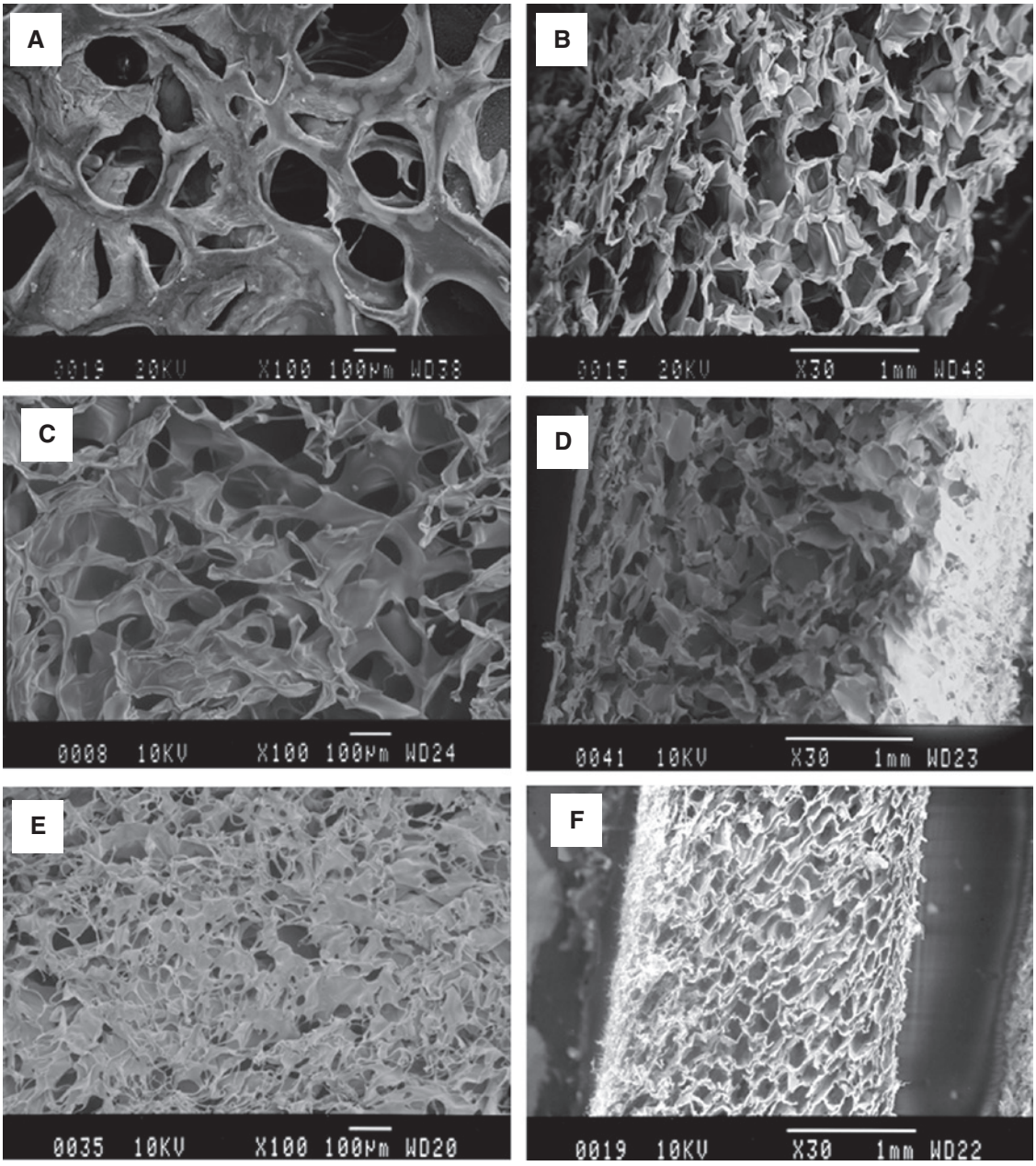

Figure 6: Surface (A, C and E) and cross section (B, D and F) scanning electron microscopy (SEM) images of alginate membrane. (A, B) 1.5%, (C, D) 2.5%, (E, F) 3.5%.

material was correlated with its hydrophilic properties. A hydrophilic material shows high water absorbency. In the molecular chain of sodium alginate, a large amount of -OH, $-COO^-$, and other strongly hydrophilic groups contributed to its strong hydrophilic properties. The capillarity of pores of the material significantly affected its water absorbency. The volume of pores was positively correlated with the water molecules. Meanwhile, the pore rate of alginate membranes prepared at different concentrations exceeded 80%. Therefore, alginate membranes have sufficient water absorbency properties. When the content of sodium alginate was increased from 1.5% to 3.5%, the network structure of the alginate membrane was comparatively compact. Moreover, the pore rate of sodium alginate decreased, which also caused a decrease in water absorbency.

The tensile strength and elongation at break of the alginate membrane increased with increasing sodium alginate content (see Figure 8). When the alginate membrane was broken by applied forces, three microscopic

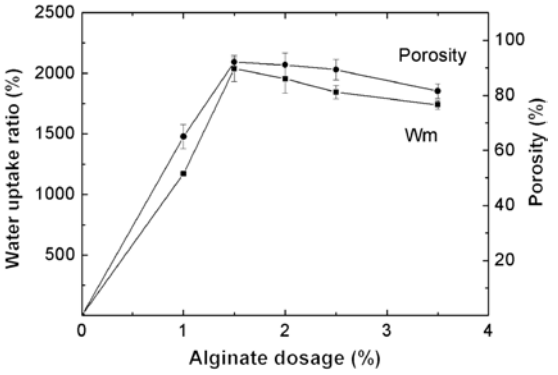

Figure 7: Effect of alginate dosage on water uptake ratio and porosity of alginate membrane.

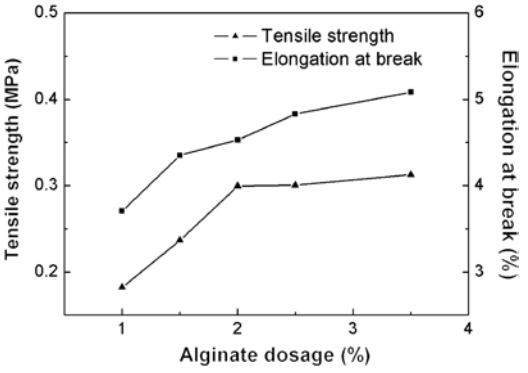

Figure 8: Effect of alginate dosage on tensile property of alginate membrane.

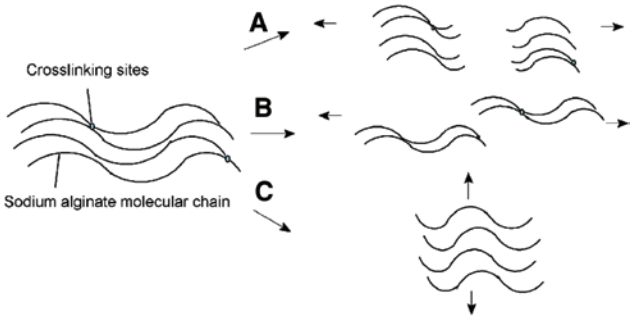

Figure 9: Microscopic changes of fracture of alginate membrane: (A) Fracture of chemical bond, (B) slippage of intermolecular, (C) Van der Waals or hydrogen bond breakage.

changes were observed [19] (see Figure 9): the alignment direction of the molecular chain of sodium alginate was parallel to the force direction, and the breakage was mainly realized by the breakage of chemical bonds or the sliding between molecules. When the molecular chain was applied with vertical force, Van der Waals or hydrogen bond breakage occurred. When the sodium alginate content was increased, the number of sodium alginate molecules in the unit volume increased. The number of hydrogen bonds between molecules also increased, which resulted in a more compact network structure. The physical intertwining of the molecular chain also hindered the sliding of the molecular chain of sodium alginate. Therefore, the tensile strength and elongation at break also increased.

4 Conclusions

Our study used sodium alginate as the raw material. After freezing and drying, the cellular alginate membrane was prepared via the crosslinking of calcium chloride. The material was white and had mutually connected pores. Our conclusions are as follows:

1. The membrane-forming property of the M_w=3.0×10^5 alginate membrane was optimum. The membrane-forming property of the material can be regulated by the molecular weight. Changing the pre-freezing temperature can regulate the pore structure to a particular degree. Pre-freezing at -10°C can result in the formation of cellular pores.
2. When the sodium alginate content was increased, the tensile strength and elongation at break of alginate membrane showed a tendency to increase. The pore rate decreased and the pore diameter was reduced when the sodium alginate content was increased. The water absorbency initially increased and subsequently decreased.

The findings of our study can provide a detailed reference that can be used to explore the effects of the preparation process on the physical and chemical properties of cellular alginate membranes. Our findings can be used as the foundation for future studies on degradable alginate membranes. The alginate porous membranes were similar to extracellular matrices of human tissues. Therefore, they would have other applications such as wound dressing, carrier of bioactive agents (proteins or small chemical drugs), and cell transplantation.

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