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Permeability of alginate/sol–gel synthesized aminopropyl-silicate/alginate membrane templated by calcium-alginate gel

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Abstract

An alginate/aminopropyl-silicate/alginate membrane was prepared on calcium-alginate gel beads via electrostatic interaction and sol–gel processes. The effects of the chemical composition and the molecular weight (MW) of the core alginate on the permeability of the alginate/aminopropyl-silicate/alginate membrane were investigated for five different types of alginates. Our data shows that an immunoisolatable MW cut-off point was achieved for the membranes formed on the alginates, MW $= 7.0 \times 10^4$ with molar ratio of M/G (mannuronic acid to guluronic acid), M/G $= 1.30$, MW $= 1.1 \times 10^5$ with M/G $= 1.30$, and MW $= 7.0 \times 10^4$ with M/G $= 0.65$. For these three membranes, the membrane templated by the alginate of MW $= 1.1 \times 10^5$ had a higher MW cut-off point than that templated by the alginate of MW $= 7.0 \times 10^4$. In addition, the MW cut-off point increased with an increase in the content of guluronic acid. The membranes formed on the alginates of MW $= 2.0 \times 10^4$ with M/G $= 1.30$, and MW $= 7.0 \times 10^4$ with M/G $= 2.25$ did not have an immunoisolatable MW cut-off point.

Keywords: Alginate; Immunoisolation membrane; Microcapsule-shaped bioartificial organ; Silicate; Sol–gel method

1. Introduction

Microencapsulation of biomolecules and living cells in calcium (Ca)-alginate gel beads coated with a semipermeable membrane has been widely investigated for industrial, pharmaceutical and medical applications [1]. The main function of core materials is to entrap biomolecules and living cells rapidly with maintaining their biological activity. The external membrane reinforces the strength of the microcapsule and inhibits the leakage of the captured biologicals. In addition, the external membrane has been applied for the immunoisolation membrane of microcapsule-shaped bioartificial organs since 1980 [2]. Many different techniques and variations of materials have been applied to this device [2–5]. The most widely investigated immunoisolation membrane is composed of the complexation between polyanionic alginate and polycationic polylysine. One of the most important functions of the membrane is to allow the free permeation of small substances such as oxygen, necessary nutrients and cell metabolites for the survival

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of the encapsulated cells. Simultaneously, the membrane must exclude immune molecules to hind the transplanted cells from an immune response of the host.

Alginates are a family of linear polysaccharides and are composed of 1,4-linked β-D-mannuronic (M) and α-L-guluronic acid (G) residues in varying chemical composition and molecular weight (MW). Gelation and crosslinking of alginate molecules mainly result from the binding of consecutive blocks of guluronic acid on individual or different alginate molecules induced by the divalent cations except magnesium. Thu et al. [6,7] reported that the chemical composition and the MW of alginate affected the permeability and the stability of the alginate/polylysine membrane.

In a previous study, an aminopropyl-silicate membrane for microcapsule-shaped bioartificial organs was developed [8]. The membrane was made from two kinds of silicon alkoxide precursors, tetramethoxysilane (TMOS; $Si(OCH_3)_4$) and 3-aminopropyltrimethoxysilane (APTrMOS; $(CH_3O)_3Si(CH_2)_3NH_2$), via a sol–gel process. The sol–gel process was performed on the Ca-alginate gel beads enclosing living mammalian cells. This membrane was composed of the electrostatic bonds between the carboxyl groups of alginate and the amino groups of aminopropyl-silicate, as well as ≡Si–O–Si≡ bonds which were induced by the sol–gel reaction. The complex with the flexible alginate polymers complemented a fragility of the sol–gel synthesized silicate. We have shown in the preceding paper [9] that the molecular permeability of the aminopropyl-silicate membrane strongly depends on the molar ratio of precursors, the sequence of addition, and the contacting time of each precursor. In addition, the pancreatic islets encapsulated in the membranes maintained their biological activity [8]. In this paper, we investigate the effect of the MW and the chemical composition of alginate on the permeability of the alginate/aminopropyl-silicate/alginate membrane.

2. Experimental

2.1. Materials

Six types of sodium alginate were used, the alginates with a intermediate guluronic acid content and MW of 5.4×10^4 (Kelton LV), molar ratio of M/G (mannuronic acid to guluronic acid) M/G) = 1.30 with MW = 2.0×10^4 (Kimica SI-L (SI-L)), M/G = 1.30 with MW = 7.0×10^4 (Kimica I-1 (I-1)), M/G = 1.30 with MW = 1.1×10^5 (Kimica I-7 (I-7)), M/G = 0.65 with MW = 7.0×10^4 (Kimica I-1G (I-1G)), and M/G = 2.25 with MW = 7.0×10^4 (Kimica I-1M (I-1M)). The values of M/G and MW of the alginates are listed in Table 1. Kelton LV was kindly provided by Kelco International (CA, USA). Kimica SI-L, I-1, I-7, I-1G and I-1M were obtained from Kimica (Tokyo, Japan). Silicon alkoxide precursors, APTrMOS and TMOS, were obtained from Tokyo Kasei (Tokyo, Japan). All chemicals were used without further purification.

Table 1
Characteristics of sodium alginate and quantities of silicate deposited on Ca-alginate gel beads

Alginate	MW	M/G ratio	Silicate deposited [g/g-dry Ca-alginate]
Kelton LV	54000	Intermediate	–
Kimitsu SI-L	20000	1.30	0.411 ± 0.018*
Kimitus I-1	70000	1.30	0.266 ± 0.006**
Kimitsu I-7	110000	1.30	0.206 ± 0.018*
Kimitus I-1G	70000	0.65	0.309 ± 0.016
Kimitsu I-1M	70000	2.25	0.217 ± 0.015**

Silicate deposited = ((dry membrane-coated gel beads) − (dry uncoated gel beads))/(dry uncoated gel beads). Error bar represents the mean ± S.E.

* $P < 0.05$ vs. I-1.

** $P < 0.001$ vs. I-1G.

2.2. Aminopropyl-silicate membrane preparation

Ca-alginate gel beads were prepared by extruding sodium alginate aqueous solution (1.5 wt.%) into 100 mM $CaCl_2$ aqueous solution. The resultant Ca-alginate gel beads (2.0 ± 0.2 mm in diameter) were rinsed with distilled water. Aminopropyl-silicate membrane was prepared by the method described earlier [8]. Briefly, the Ca-alginate gel beads were mixed in a vial with *n*-hexane and kept at 4 °C in an ice-bath. The APTrMOS was added to the *n*-hexane containing gel beads and stirred for 1 min with a vortex mixer. Then, TMOS was added and the suspension was stirred for another 1 min. Fig. 1 shows the schematic illustration of the aminopropyl-silicate membrane. The volume ratio of Ca-alginate: *n*-hexane:APTrMOS:TMOS was 10:14:0.8:0.3. All

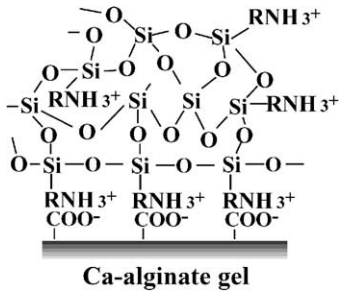

Fig. 1. Schematic illustration of the aminopropyl-silicate membrane. R = $(CH_2)_3$.

sol–gel reactions were performed at 4 °C. The addition quantity of APTrMOS (0.80 ml/10 ml of Ca-alginate) and TMOS (0.30 ml/10 ml of Ca-alginate) was chosen so that the membrane prepared at this condition on the Ca-alginate gel beads derived from Kelton LV prevented γ-globulin from diffusing through it, in our previous study [8]. After the formation of the aminopropyl-silicate membrane, the resultant gel beads were rinsed with distilled water. Subsequently, they were suspended in 0.05 wt.% sodium alginate solution for 3 min to form the external surface of an alginate layer. All the external alginate layers were prepared from Kelton LV to eliminate the effect of the external alginate layer on membrane permeability. These procedures resulted in the aminopropyl-silicate layer sandwiched between the core and the outer alginate layer.

2.3. Permeability studies

The gel beads, coated and uncoated with the aminopropyl-silicate membrane were stored for 12 h in 0.2 M Tris buffer solution (pH 7.4, 37 °C) containing 0.1 wt.% Na-azide. The Na-azide was used as a bacteriostatic agent. Time-courses of diffusion of a substance into the gel beads were measured as follows. The resultant gel beads (5 ml) were put into a gently-stirred 0.2 M Tris buffer solution (10 ml) containing 0.1 wt.% Na-azide and one of the following substances at an initial concentration (C_{L0}) of 1 mg/ml: ovalbumin (MW = 4.7×10^4) and γ-globulin (MW 1.57×10^5). Measured 20 μl of aliquots were withdrawn from the aqueous phase at various times, and concentrations of the substances at any time (C_L) were determined spectrophotometrically according to the method of Lowry et al. [10]. The measurements were carried out for 120 h, and four to six times, independent experiments were performed. Mean ± S.E. are shown in the figures.

2.4. Quantity of silicate deposited

The aminopropyl-silicate membrane-coated and the uncoated gel beads without an outer alginate layer were vacuum-dried for 2 days, after which they were weighed. The quantities of aminopropyl-silicate deposited per 1 g of Ca-alginate were calculated from the equation: (silicate deposited) = ((dry membrane-coated gel beads) − (dry uncoated gel beads))/(dry uncoated gel beads). Mean ± S.E. are shown in the figures. Statistical evaluation was performed using the two-tailed Student's unpaired *t*-test. Results were considered significant with $P < 0.05$.

3. Results and discussion

An alginate/polylysine/alginate capsule is composed of a core material of alginate and a polyanion/polycation/polyanion membrane. It has been reported that the polymer variables, such as the MW and the chemical composition of alginate affect the permeability, the stability, and the binding strength of polycation to polyanion of the alginate/polylysine/alginate membrane [6,7]. The notable difference in the membrane structure between the alginate/polylysine/alginate and the alginate/aminopropyl-silicate/alginate is as follows. The latter membrane is made up of three-dimensional ≡Si–O–Si≡ networks derived by the sol–gel process, as well as, the electrostatic bonds between carboxyl groups of alginate and amino groups of aminopropyl-silicate.

Figs. 2 and 3 show the effect of MW of the core alginate on the permeability of the alginate/aminopropyl-silicate/alginate membrane for ovalbumin and γ-globulin, respectively. The uncoated Ca-alginate gel beads prepared from the smaller alginate molecules allowed more rapid permeation for ovalbumin (Fig. 2A) and γ-globulin (Fig. 3A). Fig. 2B shows that, despite the fact that the permeation of ovalbumin into the uncoated gel beads made from I-7 was most difficult, only the membrane-coated gel beads made from I-1

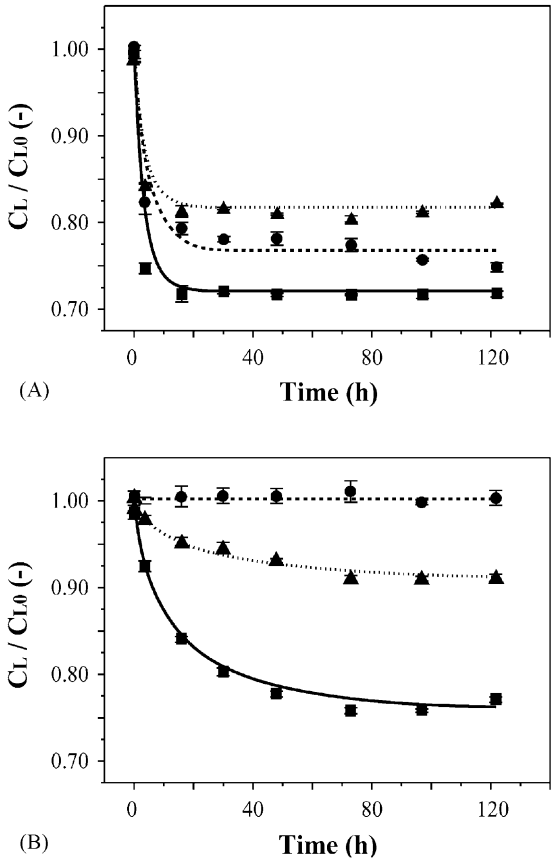

Fig. 2. Effect of the MW of alginate on time-course of diffusion of ovalbumin from 0.2 M Tris buffer solution (10 ml, pH 7.4, 37 °C) into Ca-alginate (A) and alginate/aminopropyl-silicate/alginate gel beads (B). The alginates (M/G = 1.30) with MW of 2.0×10^4 (SI-L, ■, —), 7.0×10^4 (I-1, ●, - - -), and 1.1×10^5 (I-7, ▲, · · ·) were used as a core material. Error bar represents the mean ± S.E.. Lines are the best-fit lines.

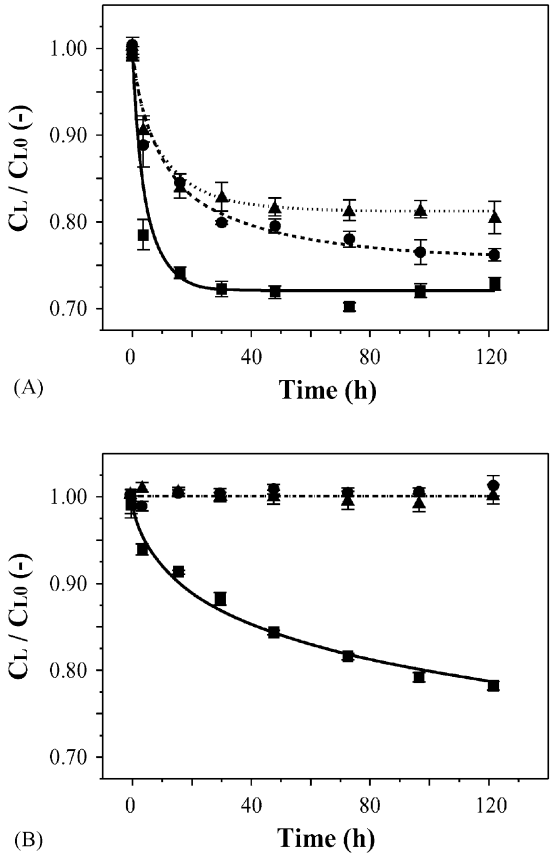

Fig. 3. Effect of MW of alginate on time-course of diffusion of γ-globulin from 0.2 M Tris buffer solution (10 ml, pH 7.4, 37 °C) into Ca-alginate (A) and alginate/aminopropyl-silicate/alginate gel beads (B). The alginates (M/G = 1.30) with MW of 2.0×10^4 (SI-L, ■, —), 7.0×10^4 (I-1, ●, - - -), and 1.1×10^5 (I-7, ▲, · · ·) were used as a core material. Error bar represents the mean ± S.E. Lines are the best-fit lines.

excluded ovalbumin. The permeation of ovalbumin into the membrane-coated gel beads made from I-7 would be attributed to the inhibition of a silica growth. The quantities of silicate deposition decreased with an increase in the MW of alginate (Table 1). The quantity of the silicate deposition on the gel made from I-1 was 0.266 ± 0.006 g/(g-dry Ca-alginate) and it was 133% of that on the gel made from I-7 (0.206 ± 0.018 g/(g-dry Ca-alginate), $P < 0.05$). This result indicates that the tighter alginate network made from the larger alginate molecules inhibited penetration of monomers and clusters into alginate polymer network. In addition, the tighter network inhibited the aggregation of these species. These inhibitions resulted in the membrane with a rougher microscopic structure than that formed on the gel beads made from the smaller alginate molecules (I-1). The mechanism for growth of the matrix in the sol–gel process is known as cluster–cluster or cluster–monomer growth, which result from aggregations of these species [11,12]. In our method, the mechanism for formation of the aminopropyl-silicate layer is considered as follows. A part of the APTrMOS added first interacted electrostatically with the carboxyl groups of the core

alginate. It was then subjected to hydrolysis and partial condensation with the retention of a large number of silanol groups. These silanol groups served as scaffold for the subsequent aminopropyl-silicate formation. Next, TMOS and the unchanged APTrMOS formed monomers and clusters. These species aggregated on the surface and in the polymer network of the Ca-alginate gel beads.

A notable result was that the quantity of silicate deposition on the gel beads made from the smallest alginate (SI-L) was the largest (0.411 g/(g-dry Ca-alginate), $P < 0.001$ versus I-1), however, the diffusion rate of ovalbumin in the gel beads was rapidest. The most rapid permeation would result from the roughest microscopic structure of the membrane. Such the formation of a rougher microscopic structure was attributed to the rougher microscopic structure of the core Ca-alginate gel. The rougher microscopic structure of the Ca-alginate gel made from the smallest alginate (SI-L) is explained from the following facts. The uncoated gel beads made from this smallest alginate exhibited the highest permeability. In addition, this alginate (SI-L) developed more fragile Ca-alginate gel beads than the others. The fragility of the gel beads required us to handle them carefully. It was reported that for the alginate with MW less than 2.4×10^5, the strength of the Ca-alginate gel was dependent on the MW [13]. Fig. 3B shows that γ-globulin (MW $= 1.57 \times 10^5$) diffused into the membrane-coated gel beads made from the smallest alginate (SI-L). The aminopropyl-silicate membranes prepared on the other gel beads (I-1 and I-7) excluded γ-globulin, i.e. these two membranes had immunoisolatable MW cut-off point. Immunoglobulin-G (MW $= 1.6 \times 10^5$) is an important antibody in the defense system against foreign antigens. The two other most important Ig classes, IgM (MW $= 9.50 \times 10^5$) and IgA (MW $= (3.0–4.0) \times 10^5$), had higher MW than that for IgG.

Figs. 4 and 5 show the effect of the content of guluronic acid of alginate on the permeability of the alginate/aminopropyl-silicate/alginate membrane. Three types of alginates, M/G $= 0.65$ (I-1G), 1.30 (I-1), and 2.25 (I-1M), having the same MW of 7.0×10^4 was used as the core material. For the three kinds of uncoated gel beads, the concentration decrease profiles of ovalbumin in the aqueous phases were almost the same (Fig. 4A). The uncoated gel

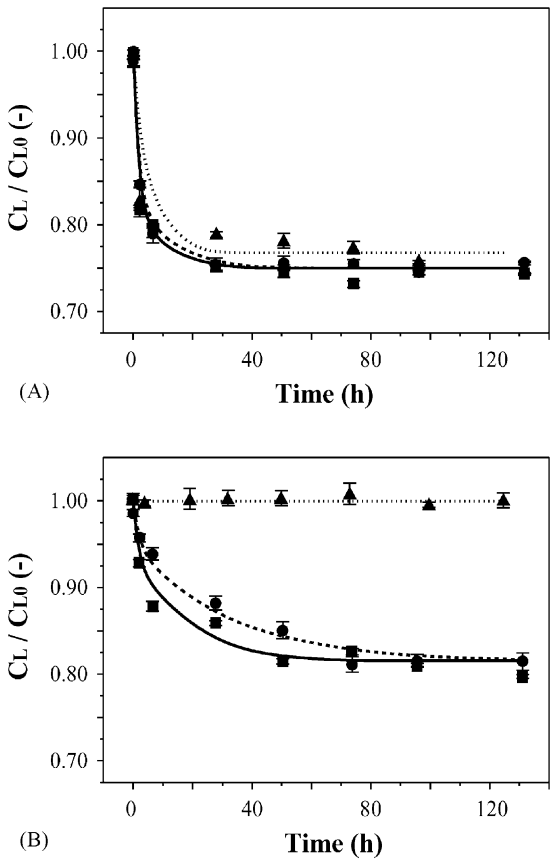

Fig. 4. Effect of the chemical composition of alginate on time-course of diffusion of ovalbumin from 0.2 M Tris buffer solution (10 ml, pH 7.4, 37 °C) into Ca-alginate (A) and alginate/aminopropyl-silicate/alginate gel beads (B). The alginates (MW $= 7.0 \times 10^4$) with M/G ratio of 0.65 (I-1G, ■, —), 1.30 (I-1, ▲, ···), and 2.25 (I-1M, ●, - - -), were used as a core material. Error bar represents the mean ± S.E. Lines are the best-fit lines.

beads rich in guluronic acid exhibited higher permeability for γ-globulin (Fig. 5A). It is known that the alginate rich in guluronic acid develops the gels having more open pore structures, which results in higher permeabilities for substances [1,7,14]. The quantity of silicate deposition on the gel beads made from I-1G was 0.309 ± 0.016 g/(g-dry Ca-alginate), and it was 142 and 116% of those on the gel beads made from I-1M (0.217 ± 0.015 g/(g-dry Ca-alginate), $P < 0.05$), and I-1 (0.266 ± 0.006 g/(g-dry Ca-alginate), $P < 0.05$), respectively (Table 1). The more open pore structure resulted from a higher content of

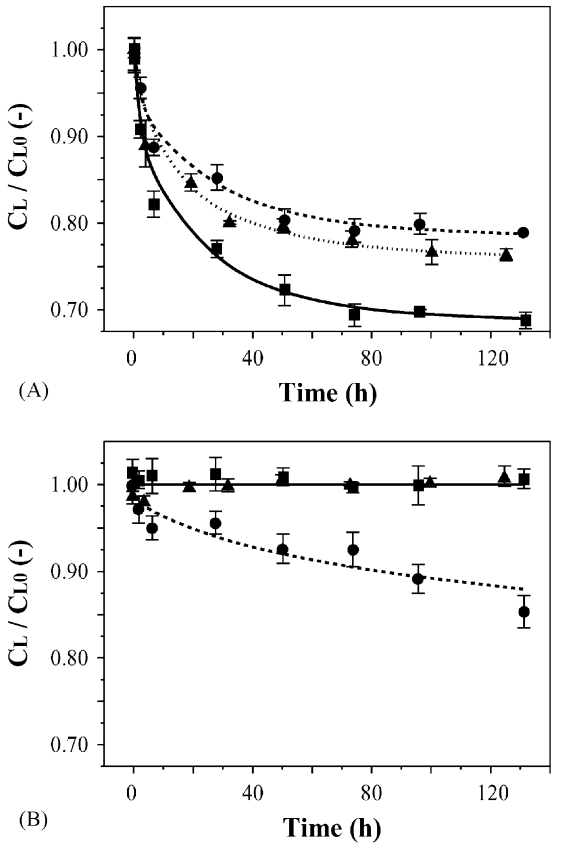

Fig. 5. Effect of the chemical composition of alginate on time-course of diffusion of γ-globulin from 0.2 M Tris buffer solution (10 ml, pH 7.4, 37 °C) into Ca-alginate (A) and alginate/aminopropyl-silicate/alginate gel beads (B). The alginates ($MW = 7.0 \times 10^4$) with M/G ratio of 0.65 (I-1G, ■, —), 1.30 (I-1, ▲, ···), and 2.25 (I-1M, ●, - - -), were used as a core material. Error bar represents the mean ± S.E. Lines are the best-fit lines.

guluronic acid enhanced the permeation and the aggregation of the monomers and the clusters in the process for the growth of the aminopropyl-silicate. It was reported that the alginates with low content of guluronic acid enhanced the binding of polylysine to alginate, owing to the higher affinity between the amino group of polylysine and the mannuronic acid of alginate [6]. The binding of APTrMOS to a core alginate would also be enhanced. However, the increase of the quantity aminopropyl-silicate deposition on the Ca-alginate with the higher content of mannuronic acid was not observed. The aminopropyl-silicate growth on the basis of penetration and aggregation of the clusters and the monomers in the alginate polymer network resulted in such differences in the quantities of silicate deposition. Fig. 4B shows that only the membrane-coated gel beads made from the alginate with M/G = 1.30 (I-1) prevented ovalbumin from permeating into them. The concentration decrease profiles of this substance for the other two membrane-coated gel beads (I-1G and I-1M) were almost the same. The diffusion profiles of ovalbumin and γ-globulin (Fig. 5B) show that the MW cut-off points of the alginate/aminopropyl-silicate/alginate membranes formed on the gel beads made from I-1 and I-1G were less than 4.7×10^4 and over the range from 4.7×10^4 to 1.57×10^5, respectively. Taking into consideration the core alginate serves as the template of the aminopropyl-silicate layer, this result can be explained by the larger pose sizes of the Ca-alginate gel made from the alginate with a higher content of guluronic acid. The membrane templated by the gel made from the alginate with lowest guluronic acid content (I-1M) exhibited the highest MW cut-off point, and it was more than 1.57×10^5. This result was attributed to the inhibition of the aminopropyl-silicate growth in the too tight Ca-alginate network, which resulted from the lower content of guluronic acid.

4. Conclusions

The aminopropyl-silicate membranes were prepared on Ca-alginate gel beads made from the alginates, which are different in MW and the guluronic acid contents. The microscopic structure of the membrane, which governs the molecular permeability was dependent on the microscopic structure of the core alginate. Except for the membrane prepared on the Ca-alginates gel beads having too rough ($MW = 2.0 \times 10^4$ with M/G = 1.30) and too tight ($MW = 7.0 \times 10^4$ with M/G = 2.25) polymer network structures, the others had immunoisolatable MW cut-off points. For the alginates with MW more than 2.0×10^4 (M/G = 1.30), the membrane prepared on the gel beads made from the larger alginate molecules exhibited a higher MW cut-off point. For the alginates with M/G value less than 1.30 ($MW = 7.0 \times 10^4$), the membrane prepared on the gel beads made from the alginate with higher content of guluronic acid exhibited higher MW cut-off point. These higher MW cut-off points of the

membranes resulted from the more open pore structures of the alginate gels, which were attributed to the smaller molecule and the lower guluronic content.

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