

The Preparation and Characterization of Fiber Reinforced Alginate Hydrogel

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ABSTRACT: To prepare fiber reinforced alginate hydrogels, calcium alginate fibers were mixed with aqueous sodium alginate solutions. Tests were carried out to assess the amount of calcium ions released from two types of alginate fibers, i.e., those made from alginate rich in guluronic acid (high G) and in mannuronic acid (high M), respectively. Experimental results showed that more calcium ions were released from the high M fibers and the hydrogel made from the high M fibers had a higher viscosity than that made from high G fibers. The water absorption and donating properties of these hydrogels

were assessed by placing them in contact with gelatine gels. The results showed that alginate hydrogels with a high alginate content are capable of absorbing water from gelatine gels, while those with a high water content are able to donate water. When silver containing particles were mixed into the alginate solution, it was also possible to prepare fiber reinforced alginate hydrogel with antimicrobial properties. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 108: 2756–2761, 2008

Key words: fibers; reinforcement; biomaterials; hydrogels

INTRODUCTION

Alginate is a polymeric acid extracted from brown seaweeds. Chemically, alginate is a copolymer of α -L-guluronic acid (G) and β -D-mannuronic acid (M), with the proportions of G and M units and the contents of GG, MM, and GM segments differing significantly for alginate extracted from different types of seaweeds.^{1,2} As a polymeric acid, alginate can form salts with metal ions of which the monovalent metal ions such as sodium and potassium form water soluble alginate salts. Most of the divalent metal ions, such as calcium and zinc, form water insoluble alginate salts. Alginate is renowned for its gel forming abilities. Upon contact with divalent metal ions, aqueous sodium alginate solutions rapidly lose their solubility and become a hydrogel.

Alginate hydrogels have been widely used in the food industry as a thickening and gelling agent. The ability of alginate to trap water has also found many applications in the biomedical industries. As a gelling agent, alginate is widely used to immobilize enzyme, cells, and yeasts.^{3–8} For example, Takei et al.³ applied a jetting technique in a liquid–liquid

co-flowing stream to the preparation of mammalian cell-enclosing calcium alginate hydrogel fibers of several hundred micrometers in cross-sectional diameter. Novikova et al. used alginate hydrogel as carriers for cells during neurotransplantation.⁶

In recent years, alginate hydrogels have found applications in the wound management industry as a novel material for the management of moisture on the wound surface.^{9,10} When applied to exuding wound, nonwoven alginate dressings absorb a large amount of exudate and form a moist gel *in situ*.^{11,12} Alginate based hydrogels can also be applied directly to relatively dry wound surfaces.^{13,14} Since the alginate hydrogel retains a high moisture content, it can help maintain a desirable moist interface between the wound and the dressing, hence facilitating cell migration and preventing dressing adherence.^{15,16}

Traditionally, alginate based hydrogels can be prepared by the introduction of calcium ions into sodium alginate solution. Since sodium alginate gels rapidly on contact with calcium ions, it is difficult to prepare a uniform alginate hydrogel by adding calcium ions into an aqueous sodium alginate solution. To prepare alginate hydrogel with a uniform gel structure, slow releasing calcium delivery systems were developed. For example, when calcium sulphate and tetrasodium pyrophosphate are mixed with sodium alginate, the calcium ions slowly release into the solution and cross-link the sodium alginate to form a uniform gel.

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TABLE I
Preparation Conditions for Fiber Reinforced Amorphous Hydrogel

Sample no.	Calcium alginate fiber mixture		Sodium alginate solution	
	Weight of calcium alginate fiber (g)	Amount of water (mL)	Weight of sodium alginate powder (g)	Amount of water (mL)
1	1	50	9	150
2	2	50	8	150
3	3	50	7	150
4	4	50	6	150
5	5	50	5	150

This article studied a new method for preparing uniform alginate hydrogels by mixing calcium alginate fibers with sodium alginate solution. In this method, calcium ions are slowly released from the fibers to crosslink the sodium alginate in solution, while at the same time, the calcium alginate fibers are converted into highly swollen sodium alginate, giving the final gel a unique fibrous texture. The alginate gel prepared in this method has the form of a viscous paste that is readily deformable, for example by molding, spreading, or extrusion through an orifice. This type of alginate fiber reinforced amorphous hydrogel can be handled considerably more easily than the traditional types of amorphous hydrogel.

EXPERIMENTAL

Materials

The sodium alginate powder used in this study was supplied by Pronova Biopolymers. It has a guluronic acid content of about 40% and a viscosity in 1% aqueous solution of about 50 cps.

Two grades of calcium alginate fibers were used. The high G calcium alginate fiber was made from a type of alginate rich in guluronic acid, while the high M calcium alginate fiber was made from a type of alginate rich in mannuronic acid. The G/M ratios for these two types of fibers were 70 : 30 and 40 : 60, respectively.

Calcium donating properties of the alginate fibers

The calcium donating properties of the alginate fibers were measured after the fibers were placed in contact with solution A as the contacting medium. The British Pharmacopoeia specifies solution A as an aqueous solution containing 142 mmol of sodium chloride and 2.5 mmol of calcium chloride. About 4 g of fibers were placed in 160 mL of solution A and the mixture was conditioned at different temperatures over different periods of time (detailed conditions are given in the Results section). At each time point, 10 mL of solution was taken out. For the high G alginate fiber, the solution was diluted by

150 times, while for the high M alginate fiber, the solution was diluted by 250 times before the calcium ion contents of the solution were measured by atomic absorption spectrometer.

Preparation of fiber reinforced amorphous hydrogel

The fiber reinforced amorphous hydrogels were prepared in a method similar to a method reported previously.¹⁷ The calcium alginate fibers were first cut to 10–15 mm length and disposed in water. Separately, sodium alginate powder was dissolved in water to form a clear and viscous solution. The two mixtures were then mixed together under vigorous stirring to form a hydrogel. Detailed preparation conditions are shown in Table I.

Preparation of alginate fiber reinforced hydrogel sheet

The preparation method for alginate fiber reinforced hydrogel sheet was similar to a previously published method.¹⁸ A piece of nonwoven calcium alginate fabric with a weight/unit area of about 100 g/m² was first cut to a round shape with a diameter of 90 mm. A 1% aqueous sodium alginate solution was prepared by mixing 5 g of sodium alginate powder with 495 mL of deionized water. An amount of sodium alginate solution was weighed into a petri dish with a diameter of 90 mm and placed on a flat surface to let the bubble evaporate. Then the nonwoven fabric was placed on top of the solution and gently immersed into the solution with a forcep. The mixture was left for 24 h, during which time, the fibers in the nonwoven fabric interacted with the sodium alginate in the solution to form a hydrogel sheet.

To prepare alginate hydrogel with antimicrobial properties, AlphaSan RC5000, which is a silver sodium hydrogen zirconium phosphate containing 3.8% by weight of silver, was mixed with the sodium alginate solution at concentrations of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0%. These solutions were then placed in contact with the calcium alginate nonwoven fabric at a solution to a fabric ratio of 40 : 1. The

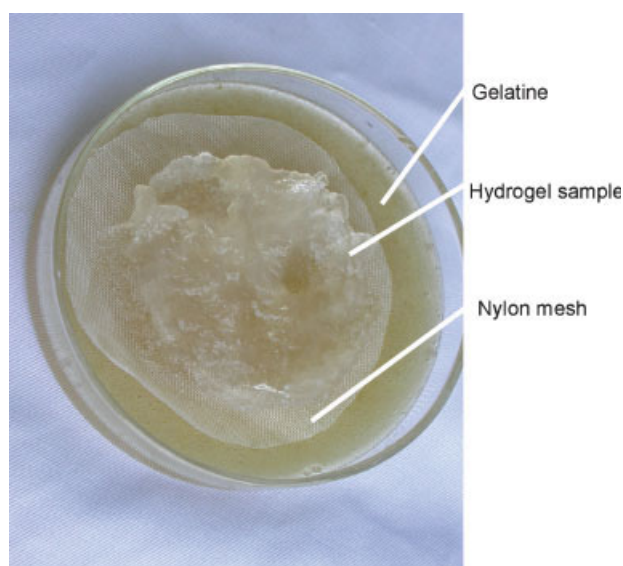


Figure 1 Schematic illustration of the fluid affinity test. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

preparation procedure is similar to the above described procedure for making hydrogel sheets.

Evaluation of the fluid affinity properties of alginate hydrogel

The fluid affinity of a hydrogel dressing determines the way in which a product might be expected to influence the moisture content of a wound and the standard test investigates its ability to donate moisture to or absorb liquid from standard substrates. About 10, 12.5, 15, and 17.5 g of gelatine were mixed with 40, 37.5, 35, and 32.5 g of solution A, respectively, and conditioned at 60°C to form clear solutions before they were poured into 90-mm diameter petri dishes and cooled at room temperature to form gelatine gels. On top of this gel was then placed a sheet of woven nylon gauze before 5 g amorphous hydrogel was placed on top of the nylon gauze. The petri dish was then sealed and placed at room temperature for 48 h. The hydrogel and the nylon mesh were then stripped off and the weight of the petri dish plus the gelatine gel was weighed. The weight change is equivalent to the amount of water donated or absorbed by the hydrogel. Figure 1 shows the schematic illustration of the fluid affinity test.

In the case of the hydrogel sheet, a piece of 2 × 3 cm gel was cut and placed directly on top of the gelatine. The water absorption and donating properties were tested in a similar procedure, except that the weight of the hydrogel sheet itself was measured at the beginning and end of the test to assess weight changes.

TABLE II
The Calcium Ion Donating Properties of High G Calcium Alginate Fibers at 20°C

Time	Calcium ion concentration in the contacting solution (mg/L)	Weight of calcium ions released as percentage of the original fiber
30 min	504.8	3.36%
1 h	527.4	3.52%
3 h	553.0	3.69%
8 h	566.6	3.78%
24 h	581.6	3.88%

Antimicrobial test

A modified version of the zone of inhibition test was used to assess the antimicrobial properties of the silver containing alginate hydrogel. Petri dishes containing the alginate hydrogels were inoculated with 0.2 mL of a log phase broth culture of *Escherichia coli*. This suspension was distributed uniformly over the surface of the plate and allowed to dry for 15 min. The plates containing the bacteria were then incubated for 24 h at 35°C. At the end of the incubation period, the plates were examined for microbial growth.

RESULTS AND DISCUSSION

The calcium ion donating properties of calcium alginate fibers

Table II shows the calcium ion concentration in the contacting solution when the high G calcium alginate fibers were placed in contact with solution A. It is clear that upon the ion exchange between the calcium ion in the fiber and sodium ions in the solution, calcium ions were released into the solution. This process is fairly rapid with the ion concentration approaching equilibrium within 30 min.

Table III shows the calcium ion donating properties of high G and high M calcium alginate fibers at 65°C. It is clear that under similar conditions, the high M alginate fibers are capable of releasing more calcium ions into the contacting solution. This is

TABLE III
The Calcium Ion Donating Properties of High G and High M Calcium Alginate Fibers at 65°C

Time	Calcium ion concentration in the contacting solution (mg/L)	
	High G fiber	High M fiber
30 min	502.8	736.5
1 h	504.2	774.2
3 h	601.4	712.5
8 h	571.6	778.8
24 h	540.8	874.0

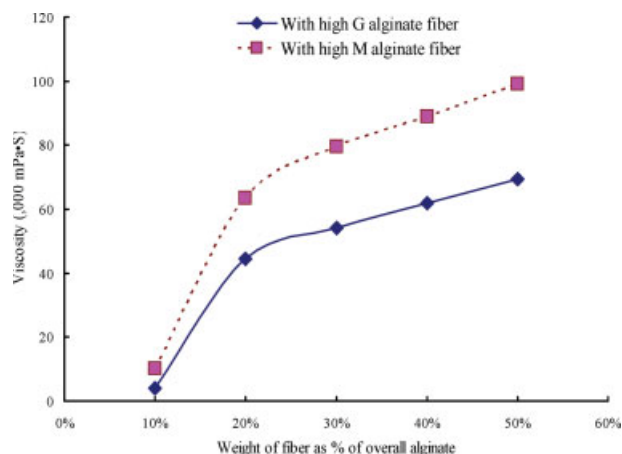


Figure 2 Viscosity of the alginate gel prepared by disposing different amounts of calcium alginate fibers into sodium alginate solutions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

because the binding between calcium ions and alginic acid is weaker in the high M alginate than in the high G alginate, where the guluronic acid units can form firm “egg box” structure with the calcium ions. When compared to Table II, it can be seen that elevated temperature had little effect on the release of calcium ions for the high G alginate fiber, suggesting that the calcium ion donating properties of the alginate fibers is influenced more by the relative proportions of guluronic and mannuronic acids of the alginate fiber.

Viscosity of the calcium alginate fiber reinforced amorphous hydrogel

Figure 2 shows the viscosity of the alginate gel prepared by disposing different amounts of calcium alginate fibers into sodium alginate solutions. In preparing these gels, the overall amount of alginate was controlled at 10 g, while the overall amount of water in each sample was 200 mL. It is clear that with the same amount of alginate material in the solution, the viscosity increases as the proportion of calcium alginate increases. With the high G calcium alginate sample, solution viscosity increased from 4.0×10^3 mPa/S when the proportion between calcium alginate and sodium alginate was 1 : 9 to 69.5×10^3 mPa/S when the proportion was 5 : 5. A similar increase was noted with the high M calcium alginate fiber sample. It is likely that as the amount of fiber increases, more calcium ions are released from the fibers to crosslink sodium alginate in the solution, hence increasing the solution viscosity. This can be verified by the difference between the gel made by using high G and high M calcium alginate fibers. Under otherwise same conditions, the viscosity of the gel made from high M calcium alginate fiber was higher than that of the high G sample, indicat-

TABLE IV
Weight Changes in Gram for the Gelatine Gel When it is in Contact with Alginate Hydrogel Reinforced with Different Amounts of High G Calcium Alginate Fibers

Weight of fiber as % of overall alginate	Gelatine concentration			
	20%	25%	30%	35%
10%	-1.835	-3.448	+3.168	+0.898
20%	-1.619	-4.758	+3.468	+3.177
30%	-1.511	-4.500	+2.733	+2.061
40%	-1.805	-5.814	+1.969	+3.060
50%	-2.738	-6.345	+2.394	+2.199

ing that high M calcium alginate fibers can donate more calcium ions to crosslink the sodium alginate.

Fluid affinity of alginate fiber reinforced amorphous hydrogels

Tables IV and V show the weight changes for the gelatine gel when it is in contact with alginate hydrogel reinforced with high G and high M calcium alginate fibers, respectively. Overall, because of the difficulty to control the accuracy of this type of test, the data obtained did not show a clear trend for different types of materials. In general, when the gelatine concentration was 20 and 25%, at the end of the test, it showed a reduction in weight, indicating the fact that water was transferred from the gelatine gel to the alginate hydrogel. When the gelatine concentration was 30 and 35%, the gelatine gel gained weight during the test, indicating its ability to absorb water from the alginate hydrogel. These results show that when the alginate fiber reinforced hydrogels are applied to wounds they can absorb water from highly exuding wounds, while they can also donate water to relatively dry wounds.

Fluid affinity of alginate fiber reinforced hydrogel sheets

Table VI shows the weight changes for the alginate hydrogel sheet when it is in contact with gelatine

TABLE V
Weight Changes in Gram for the Gelatine Gel When it is in Contact with Alginate Hydrogel Reinforced with Different Amounts of High M Calcium Alginate Fibers

Weight of fiber as % of overall alginate	Gelatine concentration			
	20%	25%	30%	35%
10%	-2.056	-4.921	+3.379	+1.262
20%	-2.189	-5.003	+2.210	+2.049
30%	-2.904	-4.672	+3.301	+2.955
40%	-2.896	-5.131	+1.448	+2.071
50%	-2.504	-5.368	+1.809	+2.547

TABLE VI
Weight Changes for the Alginate Hydrogel Sheet When it is in Contact with Gelatine Gel

Ratio between the weight of sodium alginate solution and calcium alginate nonwoven fabric	Weight gain (loss) %
30 : 1	+3.45
40 : 1	-9.57
45 : 1	-8.40
50 : 1	-7.86

gel. When the ratio between the weight of sodium alginate solution and calcium alginate nonwoven fabric was 30 : 1, the alginate hydrogel sheet had a high solid content and it had a rigid structure. During the fluid affinity test, it is able to absorb water from the gelatine gel. When the ratio was 40, 45, and

50, the hydrogels prepared had a higher water content, and they were able to donate water to the gelatine gel. From these results, it can be concluded that depending on the wetness of the wounds, alginate hydrogel sheets with different water absorption or donating properties can be prepared by controlling the ratio between the weight of the sodium alginate solution and the calcium alginate nonwoven fabric.

The antimicrobial properties of silver containing alginate hydrogel sheet

To prepare alginate hydrogel with antimicrobial properties, silver containing Alphasan RC5000 particles were mixed with the sodium alginate solution before it was combined with a piece of calcium alginate nonwoven fabric. The calcium ions released from the nonwoven fabric crosslink the sodium alginate to form a hydrogel sheet, in the meantime

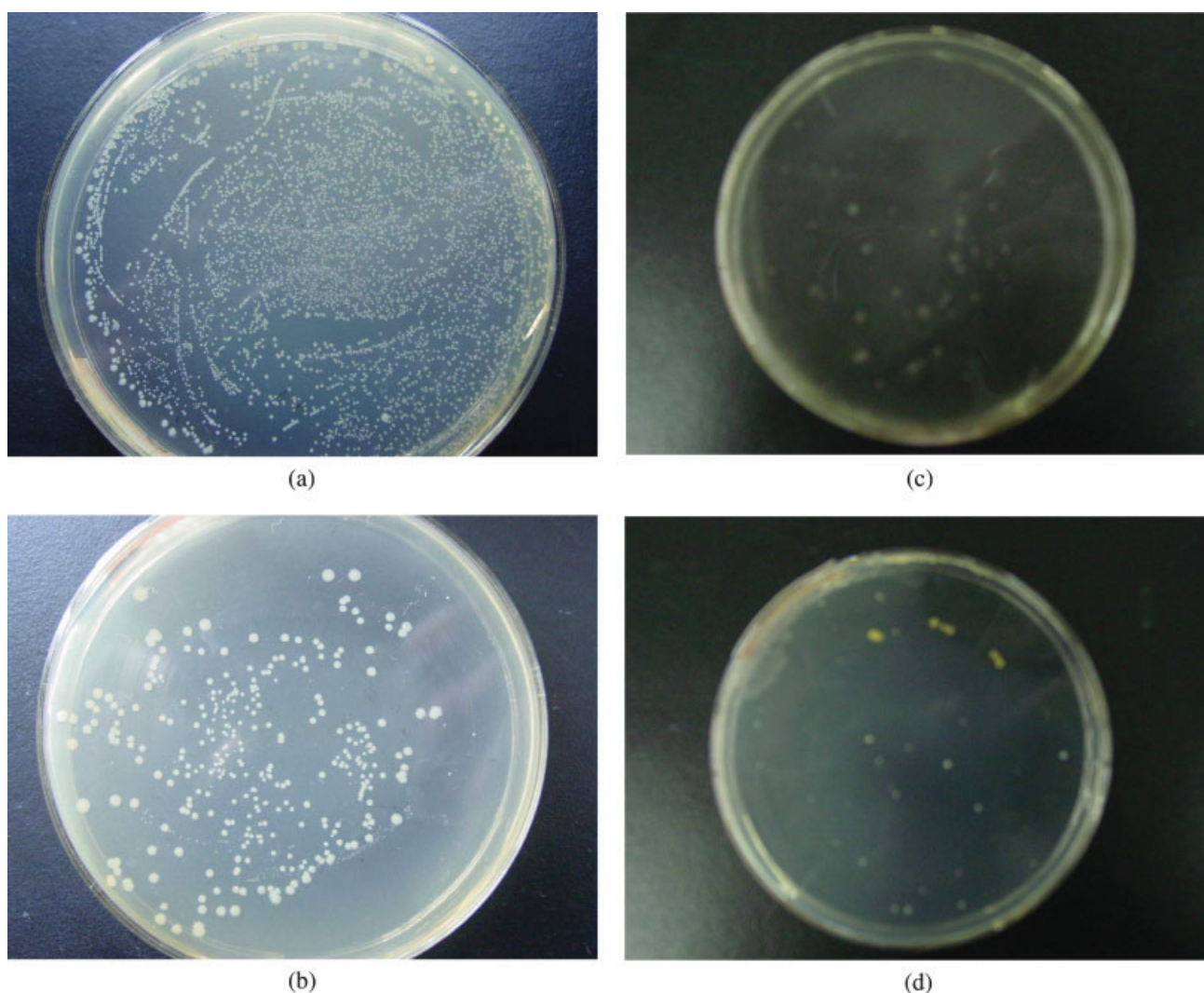


Figure 3 Schematic illustration of the effect of the addition of Alphasan RC5000 on bacteria (*E. coli*) growth, the respective addition of Alphasan RC5000 being (a) 0%, (b) 0.5%, (c) 1%, and (d) 2%. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

immobilizing the silver containing particles in the gel.

Figure 3(a–d) shows the schematic illustration of the effect of the addition of Alphasan RC5000 on the bacteria (*E Coli*) growth on alginate gel. It can be seen that when no silver containing particles were added, bacteria can rapidly multiply on the surface of the alginate gel, producing numerous coli forming units on the gel surface [see Fig. 3(a)]. Figure 3(b) showed that with the addition of 0.5% Alphasan RC5000, bacteria was still able to multiply. However, when Alphasan concentration exceeded 1%, there were few coli forming units on the gel surface, indicating the antimicrobial effect of the silver compound. These results show that when the calcium alginate fiber reinforced alginate gel is combined with the silver containing Alphasan RC5000 particles, the alginate gel has the ability to inhibit bacteria growth, and hence they can be used to help prevent wound infection.

CONCLUSIONS

This study shows that by mixing calcium alginate fibers with aqueous sodium alginate solution, it is possible to prepare alginate gel *in situ* when the calcium ions released from the fiber crosslink the sodium alginate in solution. The results showed that the viscosity of the gel increases with the increase in the proportion of calcium alginate fiber in the overall weight of the alginate. By blending silver containing

Alphasan RC5000 particles into the sodium alginate solution, it is also possible to prepare alginate fiber reinforced hydrogel with antimicrobial properties.

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