Supplementary Information

Effect of CO_2 release behavior on the crosslinking degree of alginate hydrogels prepared with $CaCO_3$ and carbonated water

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Experimental

Materials

Potassium alginate (K-3, mannuronate/guluronate ratio of 1.3, Mw = 1.79 − 2.04 × 10^6, viscosity of 1% aqueous solution at 20°C is 300 − 400 mPa·s) was supplied by Kimica Corp. (Tokyo, Japan). Calcium carbonate ($CaCO_3$) was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Bromothymol blue (BTB) was purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). SodaStream Spirit One Touch (SodaStream International Ltd., Israel) was used to produce carbonated water. The water used in this study was purified using a Milli-Q system (Nihon Millipore Co., Tokyo, Japan). All reagents were used without further purification.

Preparation of carbonated water

Carbonated water was prepared using a previously reported method.[1,2] SodaStream Spirit One Touch (SodaStream International Ltd., Israel) was used to carbonate the purified water. First, a 500-mL bottle was filled with 450 mL of purified water and cooled to approximately 6 °C. The bottle was then attached to the SodaStream Spirit One Touch. The CO_2 intensity was set to "medium" for the carbonation, and the carbonated water was used immediately in the experiments.

Preparation of Alg-gel sheet and disks

A magnetic stirrer was used to suspend $CaCO_3$ (0.10 g) in purified water (20 mL). Potassium alginate (0.40 g) was gradually added under stirring. After the potassium alginate was dissolved, 30 mL of carbonated water was added to the alginate/$CaCO_3$ solution for 10 s to prepare the hydrogels (Alg-gel). The gel precursor solution (20 mL) was dispensed into a Petri dish with a diameter of 90 mm, and an Alg-gel sheet was prepared. The thickness of the obtained gel sheet was 3.46 ± 0.26 mm, and the gelation time was <4 min.[2] For incubation on the wire mesh, the Alg-gel sheet was hollowed using a cork borer (diameter, 17.5 mm) at t = 10 min and placed on the wire mesh. In the case of incubation in a Petri dish, the Alg-gel sheet was left in the Petri dish, and the Alg-gel disk was prepared using a cork borer (diameter 17.5 mm) immediately before evaluation.

The starting time (t = 0) was when carbonated water was added. Incubation of the Alg-gel in a Petri dish and on a wire mesh was performed at 25 °C.

SEM observations of Alg-gel disks

The Alg-gel was examined via SEM. The Alg-gel disks prepared using the abovementioned method were incubated in a Petri dish for 24 h at 25 °C , and the disks were lyophilized. Subsequently, cross-sections of the Alg-gel were prepared using a razor blade. Finally, the Alg-gel cross sections were sputter-coated with gold and imaged using a JSM-6390A scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 15 kV.

Phase-contrast microscopy observations of Alg-gel

The Alg-gel was examined via phase-contrast microscopy. Alg-gel sheets prepared using the abovementioned method were incubated in a Petri dish for 24 h at 25 °C. The interior of the Alg-gel sheets was examined using an Axio Observer D1 microscope (Carl Zeiss Co., Ltd., Oberkochen, Germany).

BTB staining of Alg-gel disks

A magnetic stirrer was used to suspend $CaCO_3$ (0.10 g) in purified water (20 mL). Potassium alginate (0.40 g) was gradually added under stirring. After the potassium alginate was dissolved, 30 mL of carbonated water and 5 mL of BTB solution were added to the alginate/$CaCO_3$ solution for 10 s to prepare the hydrogels. Subsequently, 20 mL of the gel precursor solution was transferred into a Petri dish with a diameter of 90 mm, and the color changes of the Alg-gel disks incubated in the Petri dish and on the wire mesh were observed. The starting time (t = 0) was when carbonated water was added. Incubation of the Alg-gel in a Petri dish and on a wire mesh was performed at 25 °C.

pH measurement of Alg-gel disks

The surface pH values of the Alg-gel disks were measured using a previously reported method.[3] A pH meter with a flat electrode (LAQUAtwin pH-33B, Horiba, Kyoto, Japan) was employed to measure the surface pH. The Alg-gel disks were prepared using the

method mentioned above. The surface of the Alg-gel disk in contact with the atmosphere was defined as the front surface, and that in contact with the bottom of the Petri dish was defined as the back surface (Figure S2). After incubation for 10, 30, 60, 90, 150, 200, and 300 min, the pH values of the front and back surfaces of the Alg-gel disks were measured. The starting time (t = 0) was when carbonated water was added. Incubation of the Alg-gel in a Petri dish and on a wire mesh was performed at 25 °C.

Mechanical-property measurement of Alg-gel disks

The stress-strain characteristics of the prepared Alg-gel disks were measured using a creep meter (RE2-33005C(XZ), Yamaden Co., Ltd., Tokyo, Japan). An alg-gel sheet was prepared using the method mentioned above. For incubation of the Alg-gel disks on the wire mesh, the Alg-gel sheet was hollowed using a cork borer (diameter, 17.5 mm) at t = 10 min and placed on the wire mesh for 90 min to achieve complete CO_2 release and then transferred to the incubation on the Petri dish to prevent moisture loss for 22.5 h. In the case of incubation in a Petri dish, the Alg-gel sheet was left in the Petri dish for 24 h, and the Alg-gel disks were prepared using a cork borer (diameter 17.5 mm) immediately before evaluation. Their stress–strain curves were measured via compression to 87% using a plunger with a diameter of 16 mm. Young's modulus was calculated from the slope when applying a 3% compressive strain. The breaking stress was calculated from the maximum stress of the obtained stress–strain curve, and the breaking energy was calculated from the area of the obtained stress–strain curve.

Swelling measurement of Alg-gel disks

The swelling of the Alg-gel disks was determined by measuring the weight difference between the dried and swollen gels. Alg-gel disks prepared using the abovementioned method were incubated in a Petri dish and on a wire mesh for 24 h at 25 °C. The Alg-gel disks were immersed in purified water (50 mL) at approximately 25 °C for 1 d. After immersion, the surface moisture was removed, and the Alg-gel disks were weighed. Subsequently, the disks were lyophilized and weighed again. The swelling ratio was calculated according to Equation (1), where *Ws* represents the weight of the swollen gel, and W_d represents the weight of the dried gel.

$$\text{Swelling ratio } = \frac{W_s - W_d}{W_d} \quad (1)$$

Supplementary Figures and Tables

Table S1. Relationship between the concentration of calcium-binding units in alginate and $CaCO_3$ used in this study

COO^- in alginate (mmol/L)	COO^- in guluronic acid (mmol/L)	$CaCO_3$ (mmol/L)
41.2	17.9	20

The relationship between the molar concentration of calcium-binding units in alginate (concentration of carboxy groups in guluronic acid) and $CaCO_3$ in this study is shown in Table S1. Alginate forms a crosslinked structure when two COO^- of guluronic acid bond to one Ca^{2+} ion. Therefore, the present conditions contain an excess of $CaCO_3$ relative to the binding sites of the alginate, which enabled rapid gelation of the alginate gel. At longer gelation times, the difference in the crosslinked structure in the Petri dish and on the wire mesh due to the CO_2 release rate may be less pronounced.

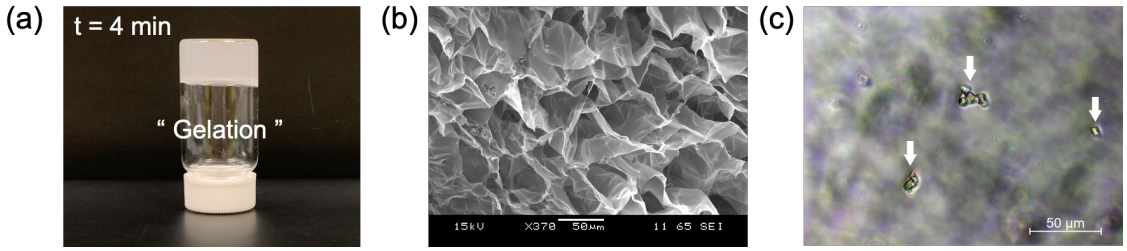

Figure S1. Preparation of an Alg-gel with carbonated water. (a) Photograph of the inversion tube test of an Alg-gel at t = 4 min. t = 0 refers to the time when carbonated water was added. (b) SEM image of an Alg-gel after 24 h of incubation in a Petri dish. (c) Phase-contrast image of an Alg-gel after 24 h of incubation in a Petri dish. The white arrows indicate $CaCO_3$ dispersed in the Alg-gel.

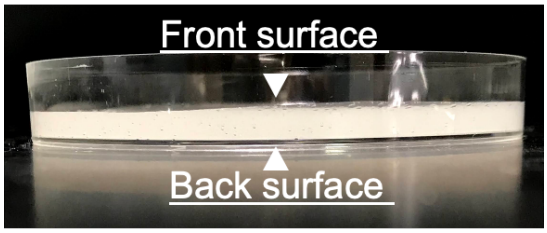

Figure S2. Definition of the Alg-gel surfaces in a Petri dish.[2]

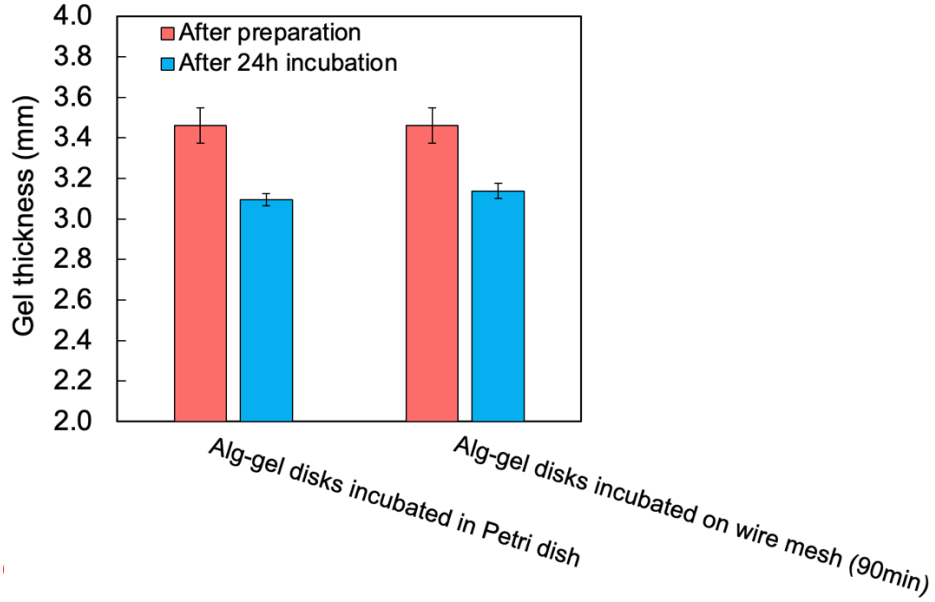

Figure S3. Changes in the thickness of the Alg-gel disks incubated in a Petri dish for 24 h and the Alg-gel disks incubated on a wire mesh for 90 min and in the Petri dish for 22.5 h. Values are expressed as means ± SE for n = 3−6.

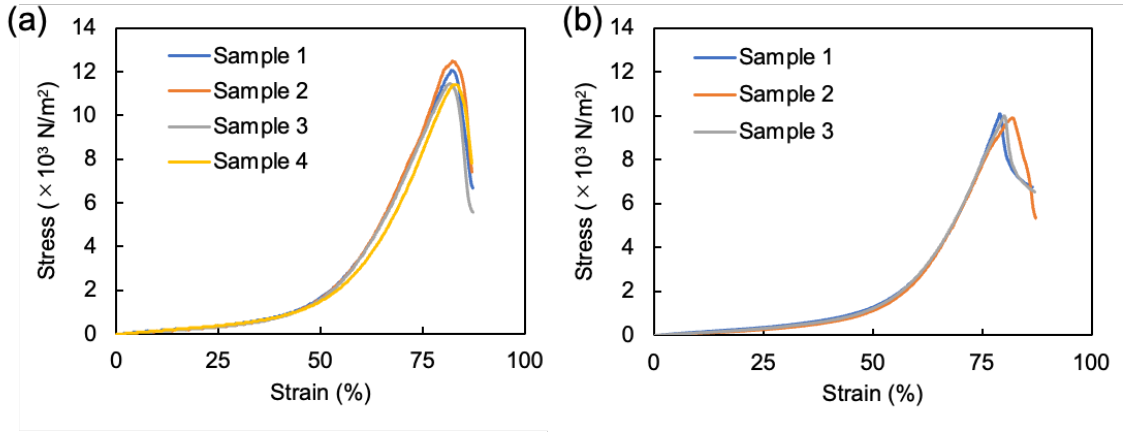

Figure S4. Stress–strain curves of Alg-gel disks incubated (a) in a Petri dish and (b) on a wire mesh.

References

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3 R. Teshima, Y. Kawano, T. Hanawa and A. Kikuchi, *Polym. Adv. Technol.*, 2020, **31**, 3032–3038.