Stability of Hydrogels Used in Cell Encapsulation: An In Vitro Comparison of Alginate and Agarose

Molly S. Shoichet,* Rebecca H. Li, Melissa L. White, and Shelley R. Winn
CytoTherapeutics, Inc., 2 Richmond Square, Providence, Rhode Island 02906

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The present studies were undertaken to evaluate the in vitro gel stability of the hydrogels alginate and agarose. Gel strength (of alginate and agarose) and protein diffusion (of alginate only) were shown to correlate with gel stability and to be useful techniques to monitor gel stability over time. The gel strengths of alginate and agarose were followed for a 90-day period using gel strength as a measure of gel stability. The gel strength of agarose diminished in the presence of cells because the cells likely interfered with the hydrogen bond formation required for agarose gelation. In the presence of cells, the gel strength of agarose decreased by an average of 25% from time 0 to 60 days, thereafter maintaining that value to 90 days. The gel strength of calcium- or barium-crosslinked alginate decreased over 90 days, with an equilibrium gel strength being achieved after 30 days. The presence of cells did not further decrease alginate gel strength. The gel strengths of calcium- and barium-crosslinked alginates were similar at 60 days—350 ± 20 g and 300 ± 60 g, respectively—indicating equivalence in their stability. The stability of calcium-crosslinked sodium alginate gels over a 60-day time period was monitored by diffusion of proteins ranging in molecular weight from 14.5 to 155 kD. From these diffusion measurements, the average pore size of the calcium-crosslinked alginate gels was estimated, using a semi-empirical model, to increase from ~176 to 289 Å over a period of 60 days.
Keywords: hydrogel • stability • gel strength • diffusion • alginate • agarose • encapsulation

INTRODUCTION

Hydrogels have been used in numerous biological technologies including gel electrophoresis and cell encapsulation. For the latter, the hydrogel may serve as a protective barrier for the cell, aiming to mimic at least some of the properties of the extracellular matrix (ECM). It has been shown that the ECM affects both cell growth and differentiation[8] as well as tissue strength and structure.[9] In cell encapsulation, the matrix material defines the extracellular environment and likely impacts cell viability, function, growth, and differentiation. The matrix may provide the required growth substrate for anchorage-dependent cells or the appropriate immobilization needed by suspension cells. The configuration and composition of the matrix material, whether of natural or synthetic origins, contributes to its effectiveness in a given application.

Numerous hydrogels have been used for cell encapsulation. For example, agarose[6] and alginate[14] have been used for the microencapsulation of islet cells for the treatment of type I diabetes. Agarose failed to provide the immuno-isolation required of xenogeneic (cross-species) transplantation whereas alginate, crosslinked with calcium and coated with poly(*l*-lysine) (PLL),[13] continues to be used. PLL improved the stability of the calcium-crosslinked alginate microbead[11] yet evoked a greater host–tissue reaction.[20] The biocompatibility of alginate microbeads was reportedly improved by coating with poly(ethylene glycol)-grafted PLL[18] or using hydroxyethyl methacrylate-grafted alginate.[19] Macroencapsulation relies on an immunoprotective and biocompatible membrane within which cells are distributed along a matrix material. The importance of the latter has been demonstrated with pheochromocytoma (PC12) cells distributed in precipitated chitosan[7] and calf adrenal chromaffin (CAC) cells immobilized in crosslinked alginate.[1]

The long-term stability of the matrix material under physiologic conditions is important to its continued use in vivo. Matrix material stability may affect continued immuno-isolation, required by microencapsulation, and safety to the host, important to both micro- and macroencapsulation. Immuno-isolation is essential for xenogeneic transplantation; encapsulated cells are protected from immunologic attack by the host, which in turn is protected from proliferation of tumorigenic cell lines that may be encapsulated. A material is considered safe for in vivo use if it is biocompatible and either biostable or biodegradable.

In the present study, the in vitro stabilities of alginate and agarose are compared, using concentrations that are compatible with encapsulation processes. Hydrogel stability, defined here in terms of the change in relative

*To whom all correspondence should be addressed. Current address: Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ontario, M5S 1A4 Canada.

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number of crosslinks and average molecular weight, was assessed by two techniques: (1) gel strength, measured with a compression test for both agarose and alginate; and (2) gel pore size for alginate, measured indirectly from diffusion rates of large proteins. For hydrogel stability as determined by gel strength, β-agarase, a degradative enzyme of agarose, was utilized to diminish the gel strength of agarose. Similarly, sodium citrate, known to decrosslink calcium alginate, was utilized to diminish its gel strength. Agarose and alginate stabilities were further measured in the presence of CAC cells relative to controls to determine the effects of cells and their secretory products on hydrogel stability. The CAC cells are of interest because they may provide analgesics for the treatment of chronic pain when using encapsulated cell therapy.[2] For hydrogel stability as determined by diffusion, the diffusion of proteins with molecular weights between 14.5 kD and 155 kD was used to track changes in pore size and distribution over time.

MATERIALS AND METHODS

Matrices

Agarose and sodium alginate (sodium salt of alginic acid) are polysaccharides derived from seaweed. Agarose, desulfonated and purified agar, was hydroxyethylated to form SeaPlaque™ agarose with a melting temperature (T_m) of 64–65°C and a gelling temperature (T_g) of 28–30°C. Agarose, a thermally reversible hydrogel, gels as a result of hydrogen bond formation, which is facilitated by aligning of the agarobiose molecule (idealized repeat unit of agarose).[10] Agarose (SeaPlaque™, FMC Bioproducts, Rockland, ME) was prepared as a 2% (w/v) solution in phosphate-buffered saline (PBS, BioWhittaker, Walkersville, MD) and sterilized by autoclaving. The agarose solution was stored refrigerated and brought into solution by microwaving prior to use. Sodium alginate is an ionic hydrogel that gels in the presence of divalent cations such as calcium and barium, forming a crosslinked ionic network. Sodium alginate (UP MVG, Pronova, Norway) was sterilized by ethylene oxide and prepared as a 2% (w/v) solution in calcium- and magnesium-free (CMF) Hank's Balanced Salt Solution (HBSS, BioWhittaker, Walkersville, MD).

Cells

Calf adrenal chromaffin (CAC) cells were isolated as previously described[2] and maintained in T75 flasks (Costar, non–tissue culture treated) in CHO-S SFM II medium (Gibco BRL, Buffalo, NY).

β-Agarase Degradation of Agarose

The 2% agarose solution was diluted with PBS (1:1, v/v) to make a 1% (w/v) solution. Five milliliters of 1% agarose was transferred by pipette to an aluminum weigh boat (Fisher, Pittsburgh, PA) and allowed to harden at room temperature for 30 min. To the hardened agarose, 1 mL of β-agarase (Calbiochem, La Jolla, CA), diluted in PBS, was added at the appropriate concentration (62.5 e.u./5 mL; 6.25 e.u./5 mL; 0.625 e.u./5 mL) and incubated at 37°C for the specified length of time (1–168 h). Controls were prepared identically to samples with the 1 mL of β-agarase solution being replaced with 1 mL of PBS. The gel strengths of experimental and control samples were measured under identical conditions and times ($n \geq 5$).

Measurement of Cell–Agarose Stability

A working stock of agarose was prepared by diluting 2% agarose with PBS (9:8, v/v). The stock solution was mixed with 2×10^6 cells/mL (4:1, v/v) and then plated in an aluminum boat (5 mL/boat) to give a 0.85% (w/v) concentration of agarose. The agarose–cell suspension was allowed to harden for 30 min at room temperature before the addition of 1 mL of medium on top. The agarose boats were then placed in Petri dishes and incubated (37°C, 5% CO_2). The medium plated on top prevented the agarose from drying out while also providing essential nutrients to the cells. Controls were treated identically but diluted with the appropriate medium in the place of cells. The agarose plates were tested over a 90-day period for stability using a gel strength compression test. Sample size for both groups was $n \geq 4$.

Sodium Citrate Decrosslinking of Alginate

The 2% (w/v) sodium alginate solution was diluted with HBSS to 1.5%. Then 3.5 mL of 1.5% sodium alginate was plated into custom-made polysulfone cups, fitted with sterile transwell 3.0-μm polycarbonate membranes (Costar, Cambridge, MA) to which 1 mL of 1% (w/v) calcium chloride was added (4 h, 37°C). The calcium chloride was removed and replaced with 1 mL of 50 m*M* sodium citrate (Aldrich, Milwaukee, WI) in PBS for up to 24 h. The gel strength was measured by compression as a function of sodium citrate exposure time ($n \geq 3$).

Influence of Crosslinker on Alginate Gel Strength and Stability

The effect of calcium chloride concentration on gel strength was also characterized. Concentrations of 1%, 1.5%, and 2% calcium chloride (w/v) was added to the 1.5% sodium alginate in the custom-made polysulfone cups as described above (1 mL for 4 h, 37°C). In addition, 1% barium chloride (w/v) was evaluated as an alternative to calcium chloride. The gel strength was measured by compression as a function of crosslinker type (cal-

cium vs. barium), concentration of calcium chloride, and exposure time ($n \geq 3$).

Measurement of Cell–Alginate Stability

The 2% (w/v) sodium alginate solution was diluted with the cell suspension in a 3:1 (v/v) ratio to give a final concentration of 1.5% alginate. Then 3.5 mL of the alginate/cell suspension was plated into the polysulfone cups and crosslinked with 1% calcium chloride as previously described. One milliliter of CHO-S SFM II medium replaced the calcium chloride. Alginate samples were incubated (37°C, 5% CO_2) until tested for gel strength by the compression test. Controls were treated identically except medium was used in the place of cells. Experimental and control samples had an $n \geq 4$.

Preparation of Alginate Gels for Protein Diffusion

Crosslinked alginate cylinders, 0.148 ± 0.018 cm in diameter (measured after equilibrium swelling), were produced by extrusion using a syringe pump/needle assembly.[12] A sodium alginate solution (1.5% w/v) was pumped into an aqueous solution of 1% $CaCl_2$/0.45% NaCl, and the cylinders were allowed to crosslink for 5 min. Single gel cylinders, of approximately 5 mL total volume, were stored in an incubator (37°C, 5% CO_2) in separate air-tight screw-top vials containing 15 mL artificial cerebrospinal fluid (ACSF) solution (with 0.05% sodium azide as a preservative) to simulate the in vivo environment that alginate may encounter (ACSF: 150 mmol Na^+, 3.0 mmol K^+, 1.4 mmol Ca^{2+}, 0.8 mmol Mg^{2+}, 1.0 mmol PO_4, 155 mmol Cl^-, pH 7.4).[21] Three vials for each time point (1, 30, and 60 days) were prepared. Diffusion of a mixture of four proteins, ranging in molecular weight from 14.5 to 155 kD, was measured by tracking the amount of each protein diffusing into the gel with time at each time point.

Protein Transport Through Calcium-Crosslinked Alginate Gels

A protein solution in ACSF consisting of 0.5 mg/mL myoglobin and 1 mg/mL each of ovalbumin, bovine serum albumin (BSA), and immunoglobulin G (IgG) was used for all diffusion studies (See Table I).[12] At each time point (1, 30, and 60 days), diffusion studies were performed by replacing the ACSF in each of the three vials containing the alginate cylinders with 15 mL of the protein solution. Samples of 150 mL of the protein solution were withdrawn to track the solute fluxes into the gel with time (sampling times were every 10 min for the first hour, followed by hourly samples for 8 h, then every 12–24 h until equilibrium was reached, for approximately 3–4 days). Protein concentrations were determined by high-performance liquid chromatography (HPLC) [with Pharmacia Superose, 10/30 column, Hitachi L-4000 ultraviolet (UV) detector at 280 nm wavelength; Waters Expert Ease version 3.1 software analysis; 50 m*M* phosphate buffer, 100 m*M* NaCl, 0.5% sodium azide, pH 7.3, mobile phase]. Diffusion experiments were performed in an incubator (37°C, 5% CO_2). The solutions were not stirred during the transport experiments because preliminary experiments (unpublished data) showed that the diffusion rates of these proteins were unaffected by vigorous stirring. Thus, the gel provides the limiting resistance to transport, indicating that boundary layer effects are not significant to this system.

Table I. Protein data.

Protein	Molecular weight (kD)	Stokes radius (Å)	D_{water} (cm^2/s), 37°C
Myoglobin	14.5	15.2	1.57×10^{-6}
Ovalbumin	43.5	27.6	1.02×10^{-6}
BSA	67	36.1	9.64×10^{-7}
IgG	155	51.3	6.29×10^{-7}

From ref. 3.

CHARACTERIZATION TECHNIQUES

Gel Strength

Gel strength was determined by measuring the break force (*g*) required to depress an anvil (diameter = 1.1 cm) through the matrix material. The force was recorded on software connected to a Vitrodyne V1000 instrument (Liveco) using either a 2000*g* or a 5000*g* load cell (Huntleigh Technologies, United Kingdom) in the compression mode.

Evaluation of Cell Viability

The viability of the cells was determined after gel testing with fluorescein diacetate (FDA, Molecular Probes, Eugene, OR) and propidium iodide (PI, Molecular Probes) which stained for viable and nonviable cells, respectively. Six micrograms of FDA and 8 μg of PI, dissolved in PBS, were added to a each cell–matrix suspension and viewed under a fluorescent microscope (Nikon, Diaphot™, Natick, MA).

Calculation of Diffusion Coefficients and Gel Pore Size (Alginate)

Diffusion coefficients were calculated using an unsteady-state mass transfer model. A simple pore size model was then used to estimate the average pore size of the gels at each time period. Detailed calculations of the transport analysis method may be found in a companion article in this issue.[12] Briefly, diffusion coef-

ficients (D) were calculated, using the method of Crank,[4] for unsteady-state diffusion from a stirred solution of limited volume into a solid cylinder of length L and radius r where $L \gg r$ and D is assumed constant. The pore size of the alginate gel was determined by the semiempirical expression (1) commonly used to describe diffusion of solutes in porous materials[16]:

$$\frac{D^i_{\text{gel}}}{D^i_{\text{water}}} = \left(1 - \frac{d}{\delta_p}\right)^2 \left\{1 - 2.104\left(\frac{d}{\delta_p}\right) + 2.09\left(\frac{d}{\delta_p}\right)^3 - 0.95\left(\frac{d}{\delta_p}\right)^3\right\} \quad (1)$$

where D^i_{gel} is the diffusion coefficient of solute i in the gel at 37°C; D^i_{water} is the diffusion coefficient of solute i in water at 37°C; d is the hydrodynamic diameter of solute i; and δ_p is the average pore diameter of the material. Here, δ_p was calculated by fitting the experimentally measured D_{gel} values for each protein with δ_p as the adjustable parameter and obtaining the best fit.[17]

Data Analyses

Overall effects were assessed utilizing the appropriate analysis of variance (ANOVA). Data are presented in the text and figures as the mean ± standard deviation (SD); statistical significance was established at $p < 0.05$.

RESULTS

Gel Strength

Agarose

The effects of both β-agarase enzyme concentration and time of interaction with agarose were studied in terms of gel strength. Figure 1 shows the gel strengths (mean ± SD) of agarose gels that were exposed to the β-agarase enzyme at specific concentrations (62.5, 6.25, or 0.625 enzyme units/5 mL agarose) and time periods (1–168 h). The controls had buffered saline solution in place of the enzyme ($n \geq 5$). The agarose controls showed consistent gel strengths between 200 and 250g, whereas the β-agarase-treated agarose gels had decreased gel strengths over time to one-tenth of their original value. Agarose exposed to 62.5 e.u./5 mL β-agarase decreased to 26 ± 3g, whereas that exposed to 0.625 units/5 mL decreased to 54 ± 5g. The higher concentration of β-agarase showed the greatest rate of agarose degradation and the least gel strength. A one-way ANOVA conducted on the differences between control and the β-agarase-treated agarose gel strengths were statistically significant for all time points and treatments ($p < 0.05$) with the exception of $t = 2$ h for the 0.625 e.u./5 mL treatment group.

The gel strength of agarose was studied in the presence of CAC cells over 90 days. Figure 2 shows the gel strength (mean ± SD) of agarose-containing CAC cells with respect to controls over 90 days. The gel strength of agarose controls decreased from 5 (173 ± 10g) to 30 days (137 ± 7g) but was constant between 30, 60 (132 ± 24g), and 90 days (139 ± 26g). The difference in gel strength for agarose controls from 5 to 30 days was statistically significant ($p < 0.05$), while no change was observed between 30, 60, and 90 days ($p > 0.05$). The gel strength of agarose decreased in samples containing CAC cells between 5 (100 ± 4g) and 30 days (91 ± 5g). CAC cell-containing agarose gels showed decreased gel strengths between 30 and 60 days (71 ± 4) or a further 22% decrease. The decrease in gel strength observed with agarose/CAC cells is statistically significant ($p < 0.05$). The gel strength of agarose/CAC cells was unchanged between 60 (71 ± 4g) and 90 days ($p > 0.05$). A one-way analysis of variance (ANOVA) conducted on the differences between the control and agarose/CAC cell treatment groups was statistically sig-

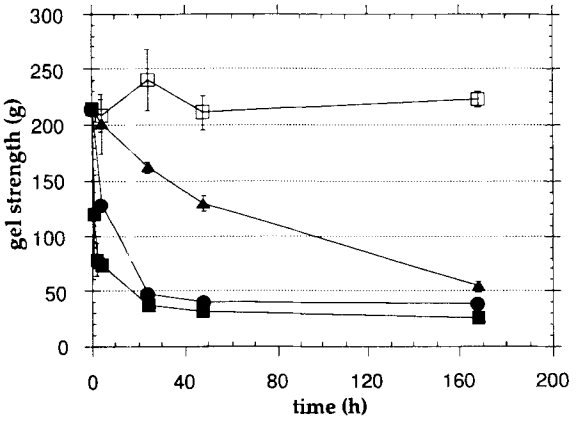

Figure 1. Degradation of agarose with β-agarose showed decreased gel strength (mean ± SD) over time and with increased β-agarase concentration: (■) 62.5 e.u.; (●) 6.25 e.u.; (▲) 0.625 e.u.; (□) controls (no enzyme) ($n = 5$).

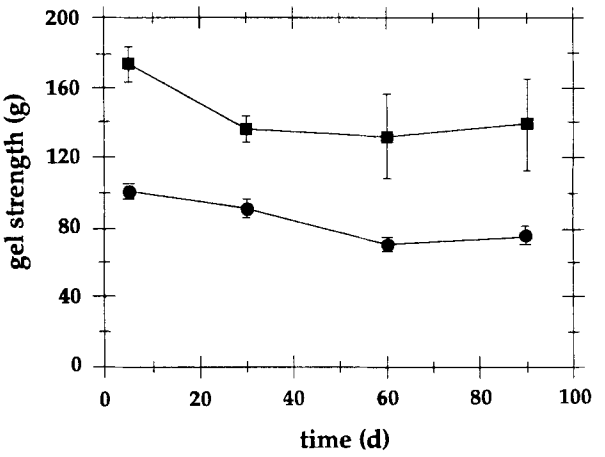

Figure 2. CAC cell-containing agarose gels (●) showed decreased gel strength (mean ± SD) with respect to controls (■).

nificant ($p < 0.05$). By FDA/PI staining after 90 days, the CAC cell viability was estimated at 70 ± 10%.

Alginate

By measuring the gel strength of both sides of the crosslinked disc, it was determined that 4 h was required to achieve a homogeneous crosslinked alginate disc with a 1% calcium chloride solution. Prior to measuring stability of crosslinked gels, it was found that the gel strength of alginate was dependent upon the concentration of calcium chloride used: for 1% calcium chloride, alginate had a gel strength of 840 ± 70*g*; for 1.5% calcium chloride, alginate had a gel strength of 1480 ± 180*g*; and for 2% calcium chloride, alginate had a gel strength of 2220 ± 125*g*. Sodium alginate (1.5% w/v) was also crosslinked with a 1% barium chloride solution for 4 h and found to have a gel strength of 626 ± 63*g*.

A similar study to that of β-agarase enzyme on agarose was performed to correlate calcium-crosslinked alginate gel stability with gel strength. The gel strength of calcium alginate (using 1% calcium chloride) was measured as a function of time in the presence of 50 m*M* sodium citrate, as shown in Figure 3 ($n \geq 3$/time point). The gel strength of alginate decreased with exposure to sodium citrate, from an initial 1061 ± 53*g* to 385 ± 21 at 0.5 h. By 1.25 h, the gel strength had diminished to 227 ± 27*g*; the further decrease to 137 ± 17*g* that was observed at $t = 6$ h remained consistent out to 24 h. An ANOVA revealed that the difference in initial gel strength was statistically significant with the treatments at all time points analyzed ($p < 0.05$).

The stability of calcium-crosslinked alginate was studied over 90 days in the presence of CAC cells relative to controls, as shown in Figure 4 (mean ± SD). The average gel strength of alginate has been shown to decrease by approximately 40% within the first 9 days (from 834 ± 78*g* to 561 ± 44*g*). In the present studies, the gel strength

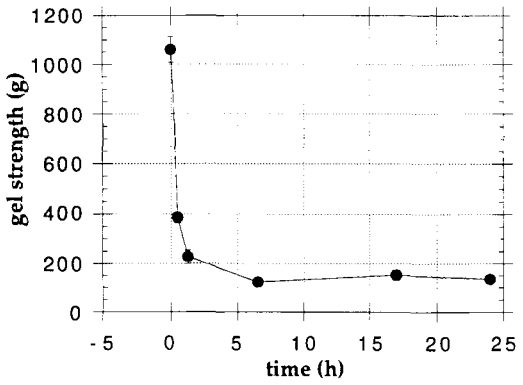

Figure 3. Gel strength of calcium-crosslinked alginate decreased as a function of sodium citrate exposure time as a result of decrosslinking.

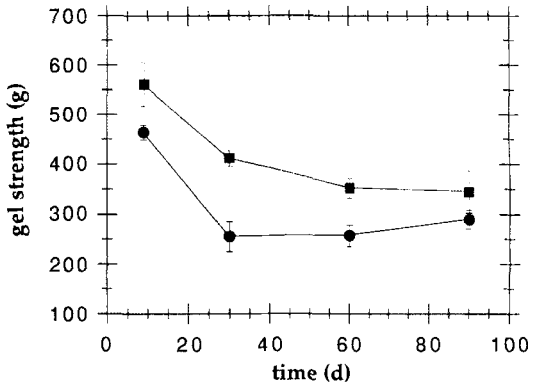

Figure 4. CAC cell-containing calcium-crosslinked alginate gels (●) showed decreased gel strength with respect to controls (■) initially; in both cases, a significant decrease over time was observed in gel strength.

of controls decreased from 561 ± 44*g* at day 9 to 412 ± 16*g* by 30 days ($p < 0.05$). A further decrease was observed from 30 to 60 days (353 ± 19*g*) that was consistent out to 90 days (345 ± 42*g*). The average gel strength of the CAC cell-containing group was 464 ± 15*g* at 9 days and decreased to 255 ± 30*g* by 30 days ($p < 0.05$). However, no statistically significant change was observed thereafter at 60 days (257 ± 22*g*) and 90 days (290 ± 19*g*). Using the fluorescent viability/nonviability stain (FDA/PI), the cells showed 80 ± 10% cell viability.

The gel strength of alginate was followed over 21 days on samples where the medium was changed 2–3 times/week vs. those where the medium was not changed yet was maintained at a specific volume. The gel strength of alginate decreased from 791 ± 85*g* to 511 ± 71*g* when the medium was changed and to 773 ± 79*g* when the medium was not changed.

The gel strength of barium-crosslinked alginate (1% barium chloride) was measured over 60 days in the presence of CAC cells. The gel strength of barium-crosslinked alginate decreased from the initial value of 626 ± 63*g* to 304 ± 61*g* after 60 days ($p < 0.05$). The gel strength of barium-crosslinked alginate did not decrease significantly ($p > 0.05$) between 30 (325 ± 69*g*) and 60 (304 ± 61*g*) days. In the presence of CAC cells, the gel strength was 279 ± 68*g* after 30 days and 339 ± 70*g* after 60 days.

Protein Diffusion

From the best fits of Equation (1), the average pore size of the calcium alginate gels increased over a 60-day period from 176.2 Å (at 9 days) to 208.5 Å (at 30 days) to 288.1 Å (at 60 days). Table I shows the protein parameters used in Equation (1) to calculate pore sizes.

Figure 5 shows the diffusion coefficients (mean ± SD) for each protein. The results show an insignificant change in the diffusion of the largest protein, IgG, over 60 days; a significant ($p < 0.05$) increase in the diffusion

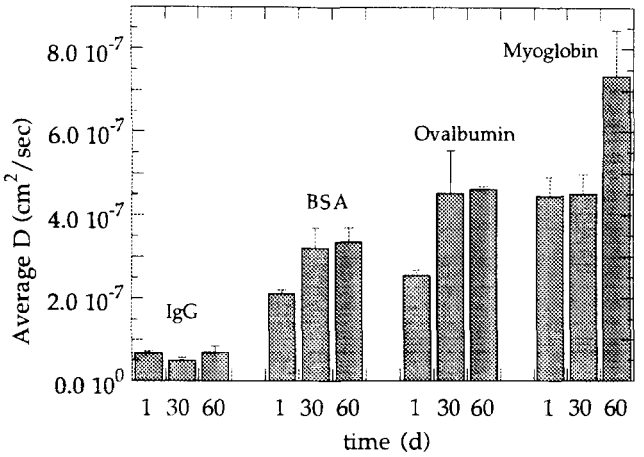

Figure 5. Protein diffusion coefficients (D) were calculated at each time point (1, 30, and 60 days).

of BSA (by 40 ± 5%) and ovalbumin (by 55 ± 3%) between 1 and 30 days; and a significant ($p < 0.05$) increase in the diffusion of myoglobin (by 48 ± 7%) between 30 and 60 days.

DISCUSSION

The decrease observed (in Fig. 1) in agarose gel strength with increased β-agarase concentration indicates that gel strength correlates with, and can be used as a measure of, gel stability. β-Agarase, from *Pseudomonas atlantica*, cleaves the carbohydrate bonds of agarose, thereby converting unsubstituted neoagarobiose [3,6-anhydro-a-L-galactopyranosyl-(1,3)-D-galactose] to neoagaro-oligosaccharides.[5] Similarly, as shown in Figure 3, increased exposure of calcium-crosslinked alginate to sodium citrate results in decreased gel strength because sodium citrate chelates calcium, thereby decrosslinking calcium alginate. The correlation of decreased gel strength with decrosslinking enables the correlation to be made between gel strength and gel stability for alginate.

For the model system where the effects of β-agarase on agarose gel strength were followed, 1% agarose was used; for subsequent studies, 0.85% agarose was used. While the difference in agarose concentration affected its gel strength, it did not affect the relative comparisons made. As shown in Figure 2, the initial gel strength of agarose controls was significantly higher than that of agarose–cell samples because the cells likely interrupted the hydrogen bond formation between aligned polymer chains of agarose. The relative change in agarose gel strength between 5 and 90 days was approximately 20% and 25% for the control and CAC cell groups, respectively. Hydrolytic degradation may account partially for the observed decrease. Degradation resulting from the release of cell secretory products may account for the further decrease in gel strength observed in agarose–cell samples at 60 days. The plateau of gel strength reached between 60 and 90 days in the presence of CAC cells may indicate that either (1) the CAC cell secretory products cannot further degrade agarose or (2) the CAC cells stop secreting "degradation" products while maintaining their viability.

The decrease in alginate gel strength observed in Figure 4 may result from either degradation or decrosslinking by calcium diffusion out of the matrix. Unlike agarose, which gels thermally upon hydrogen bond formation, alginate gels by divalent ionic crosslinks. In contrast to the effect of cells on agarose gel formation, where a 45% decrease was observed between control and experimental samples, cells had a limited affect on initial alginate gel formation. Although the gel strength of CAC cell-containing alginate decreased more rapidly than controls between 9 and 30 days, both had similar gel strengths at 90 days. This indicates that the decrease in alginate gel strength may have resulted from decrosslinking as opposed to degradation. The gel strength of alginate decreased, over 21 days, when the medium was changed, relative to controls, when the medium was not changed. This indicates that the gel strength of alginate decreased as a result of decrosslinking, or calcium diffuson out of the matrix.

A similar trend in decreased gel strength was observed for barium-crosslinked alginate: (1) initial gel strength decreased by 51% within the first 30 days and (2) CAC cells did not further decrease the gel strength of barium alginate with respect to controls. While barium diffusion out of the alginate gel likely accounted for its decreased gel strength, degradation cannot be eliminated and may contribute to the resultant decreased gel strength.

Another method to follow alginate stability involves monitoring changes in pore size and distribution which can be estimated by calculating protein diffusion coefficients. Diffusion rates through gels have been shown to be a function of the degree of crosslinking, where the diffusion coefficient increases as crosslinking density decreases.[15] Solutes (i.e., proteins) can be classified in relationship to the gel pore size as "small," "medium," or "large": small solutes pass relatively unhindered through the gel; medium solutes experience some hindrance to transport; and large solutes are severely hindered in their transport through the gel.

The change in protein diffusion rates over time can be described in terms of the stability of the calcium-alginate ionic bond. As the number of these crosslinks decreases by calcium ion diffusion out of the matrix, the average pore size increases and the pore size distribution shifts to larger pores. In this case, diffusion rates of the previously hindered medium-sized solutes increases while that of small solutes remains unchanged until the population of pores through which they can diffuse increases sufficiently to affect their transport. The diffusion rates of large solutes remain constant until the average pore size increases significantly.

Changes in the diffusion rate of hindered solutes are indicative of changes in gel structure and thus gel stability.

The data in Figure 5 show that the diffusion rates of medium solutes (ovalbumin and BSA) increased within the first 30 days and then remained constant over the next 30 days, when the population of pores through which these solutes could diffuse reached a plateau; that of the large solute (IgG) remained unchanged over 60 days; and that of the small solute (myoglobin) increased between 30 and 60 days only. Although there was a general shift toward larger pores in the pore size distribution, this shift affected neither the small solutes until after 30 days nor the larger solutes within the time frame of this experiment (60 days). Medium solutes, hindered in their diffusion by the greatest number of pores, were most affected by the distribution shift toward larger pores (assuming a Gaussian distribution in pore size for these gels). Small solutes, hindered by the fewest number of pores, required a more substantial shift in pore size distribution for the diffusion rate to change significantly (i.e., after 30 days). Estimations of the degree to which average pore size is changing with time may affect cell encapsulation applications, where, in the case of microencapsulation, the gel provides immunoisolation.

The ratio of gel volume to surrounding reservoir volume was not considered in this analysis and likely affects the rate of decrosslinking of calcium alginate gels. While it was expedient to use a ratio of 1:3 for gel-to-reservoir volumes, a larger ratio (such as found in vivo) may result in the increased diffusion rate of calcium through the gels, a shift to larger pore sizes, and a decreased number of calcium alginate ionic bonds.

CONCLUSIONS

The gel stabilities of agarose and alginate, with and without CAC cells, were monitored by gel strength and, additionally, alginate stability was assessed by protein diffusion. The initial gel strength of agarose, in contrast to alginate, diminished in the presence of CAC cells. Both techniques indicated that the number of calcium alginate bonds decreased with time, resulting in decreased gel strength and increased permeability of medium-sized solutes. Gel strength and protein diffusion were used in parallel to elucidate different aspects of gel stability. Gel strength is important when the gel is required to impart mechanical integrity to an implant material. The compression test used to monitor gel strength is easy to use and is particularly conducive to following the effect of living cells on matrix stability. Protein diffusion through the gel is important for immuno-isolatory applications and for estimation of average pore size of the gel. The cylindrical geometry and crosslinking conditions used mimic those of the macroencapsulated implant configuration.

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