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pH-Responsive Jello: Gelatin Gels Containing Fatty Acid Vesicles†

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We describe a new way to impart pH-responsive properties to gels of biopolymers such as gelatin. This approach involves the embedding of pH-sensitive nanosized vesicles within the gel. The vesicles employed here are those of sodium oleate (NaOA), a fatty-acid-based amphiphile with a single C_{18} tail. In aqueous solution, NaOA undergoes a transition from vesicles at a pH ~8 to micelles at a pH higher than ~10. Here, we combine NaOA and gelatin at pH 8.3 to create a vesicle-loaded gel and then bring the gel in contact with a pH 10 buffer solution. As the buffer diffuses into the gel, the vesicles within the gel get transformed into micelles. Accordingly, a vesicle−micelle front moves through the gel, and this can be visually identified by the difference in turbidity between the two regions. Vesicle disruption can also be done in a spatially selective manner to create micelle-rich domains within a vesicle-loaded gel. A possible application of the above approach is in the area of pH-dependent controlled release. A vesicle-to-micelle transition releases hydrophilic solutes encapsulated within the vesicles into the bulk gel, and in turn these solutes can rapidly diffuse out of the gel into the external bath. Experiments with calcein dye confirm this concept and show that we can indeed use the pH in the bath to tune the release rate of solutes from vesicle-loaded gels.

1. Introduction

In recent years, there has been immense interest in creating smart materials, i.e., materials that can change their properties in response to external stimuli such as light, temperature, pH, or biological targets.[1−4] In particular, stimuli-responsive polymer hydrogels have attracted much attention, and a stimulus of interest in this context has been pH.[1−3] Polymers having ionizable groups (acid or base) tend to form gels with pH-responsive swelling properties. Such gels generally tend to be swollen when the groups are ionized, while they revert to a collapsed or shrunken state when the same groups lose their charge. Examples include gels of poly(acrylic acid), poly (methacrylic acid), etc., and their copolymers, and these have proven attractive for controlled release and drug delivery applications.[1−3]

An alternate way to impart pH-dependent properties to polymer gels is by embedding pH-responsive nanoparticles or nanostructures in their interior.[4] In this scenario, the polymeric framework itself is unaffected by pH (e.g., there is no change in the degree of gel swelling with pH). This approach may be attractive for a variety of reasons. For instance, one may wish to modulate the release of a drug from a gel while maintaining a constant gel volume. Second, by embedding nanostructures, *pH-responsiveness can be conferred to materials that by themselves do not have such properties*. For example, consider hydrogels of gelatin and poly(*N*-isopropylacrylamide) (NIPAAm).[2,5] Both gels have thermoresponsive properties: gelatin gels melt upon heating while NIPAAm gels shrink when heated beyond a critical temperature. While these polymers are workhorses in many applications, they are not pH-responsive (at least not at moderate pH values). One could modify the chemistry of these polymers to make the gels pH-sensitive, but that is generally a tedious process. The addition of nanostructures to impart pH-responsiveness is generally a much simpler alternative.

In this study, we explore the addition of pH-responsive *vesicles* into gelatin gels and investigate the resulting vesicle−gel hybrids. Unlike "hard" nanoparticles, vesicles (or liposomes) are "soft" self-assembled structures formed from lipids or surfactants.[6] Unilamellar vesicles of ca. 100 nm diameter can be essentially considered as nanocontainers, with a bilayer membrane enclosing an aqueous core.[6−8] Vesicles thus have the ability to encapsulate hydrophilic solutes in their core, and for this reason they are of great interest for drug delivery applications.[6−8] In the present context, we are interested in embedding vesicles within a polymer hydrogel network. Vesicle−gel hybrids have been investigated in the past by several researchers.[9−14] The motivation for past studies has

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been the use of these hybrid materials in tissue engineering or drug delivery. For example, drug delivery from a vesicle-loaded gel has been shown to occur in a prolonged manner compared to that from either the vesicles themselves or the bare gel alone.[9−14] To our knowledge though, pH-responsive vesicles have not been combined with hydrogels previously—this is the first study to explore such a combination.

The pH-responsive vesicles we have chosen to examine are those made from sodium oleate (NaOA), the sodium salt of a single-tailed fatty acid, oleic acid. It has been known for many years that fatty acids can self-assemble into unilamellar vesicles at pH values close to their pK_a.[15−19] Fatty acid vesicles have been investigated particularly by researchers in the field of prebiotic chemistry—these researchers believe that fatty acids and by extension their vesicles may have been components in the prebiotic soup of ancient earth (indeed, fatty acids have even been isolated from meteorites).[17−19] In the present study, we exploit the well-known pH-dependence of fatty acid self-assembly.[15,16] For example, NaOA forms vesicles at a pH around its pK_a of 8.3, but these vesicles transform into spherical micelles at pH values much higher than the pK_a, e.g., at pH >10 (see the phase diagram in Figure 1).[16] We were interested to see whether such a pH-induced vesicle-to-micelle transition would occur even if the NaOA vesicles were entrapped within a gel. As the results in this paper show, this is indeed the case. The above concept can be extended to other pH-dependent vesicles (e.g., those formed from certain lipids[20]). Also, instead of gelatin, a variety of polymer and biopolymer-based hydrogels can also be used as the matrix for the vesicles.[3,21]

Finally, it is worthwhile to discuss some applications for gels loaded with pH-responsive vesicles. An obvious one would be in pH-controlled release of hydrophilic solutes.[1−3] Consider solutes encapsulated within the vesicles, which in turn are loaded into a gel. If such a gel is immersed in an aqueous bath, the solute will slowly diffuse out of the gel. We can then use the pH in the bath to modulate the solute release rate. For example, at a pH corresponding to intact vesicles, the solute will face two barriers to transport: one from the vesicle bilayer and the other from the gel matrix.[9−14] On the other hand, consider a pH at which the vesicles are disrupted into micelles: in this case, the solute will only face resistance from the gel matrix and should therefore be able to diffuse out faster. In our studies, we have tested the above concept using a dye, calcein, as a model solute, and our findings confirm a pH-tunable release rate.

A vesicle-loaded gel could also serve as a simplistic model for a functional organ, with the vesicles as the cellular component and the gel as the extracellular matrix.[3,22] The overall functionality of an organ depends on the ability of cells to communicate with neighboring cells.[22] Cells of different types may be localized at different parts of the organ; e.g., they may be organized into distinct layers, with their location being strongly correlated with cellular function. In this context, it is useful to be able to dictate the spatial organization of vesicles within a gel, i.e., to have only certain regions filled with vesicles while adjoining regions would not contain any. We show that pH-responsive vesicles offer one way to accomplish such a design: specifically, we demonstrate how vesicle-to-micelle transitions can be achieved in localized regions within a gel. This opens up the prospect of creating a "biomimetic organ" by placing pockets of vesicles loaded with specific molecules at prescribed locations within a gel matrix.

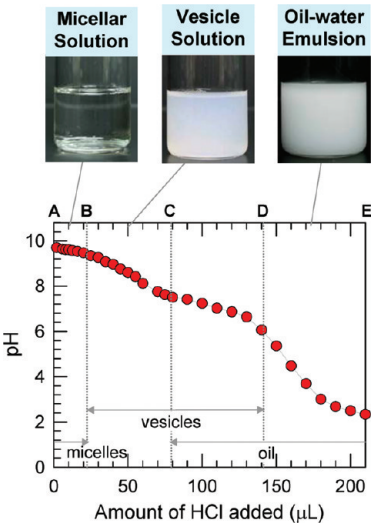

Figure 1. Photographs and titration curve for 1% NaOA at 25 °C. Increasing amounts of 1 M HCl are added to a micellar solution of NaOA (pH 10), and the corresponding solution pH at equilibrium is shown in the plot. Structural assignments are done in accordance with ref 16. Photographs of samples corresponding to different regions of the plot are shown above.

2. Experimental Section

Materials. Sodium oleate (NaOA) (note: oleate = C18 chain with a 9-cis unsaturation) was purchased from TCI. Gelatin (Type A, from porcine skin, ~300 Bloom), calcein, Triton-X100, and Tris-base were purchased from Sigma. Carbonate buffer solution (potassium carbonate−potassium borate−potassium hydroxide) corresponding to a pH of 10 was purchased from Fisher Scientific. All experiments were performed using deionized (DI) water.

Preparation of Vesicles and Vesicle-Loaded Gels. 1 wt % (32.8 mM) NaOA was dissolved in DI water by heating for 2−3 h at 65 °C. The pH of the solution was then adjusted to ~8.5 by dropwise addition of 1 M HCl. In order to generate unilamellar vesicles of consistent size, the vesicle solution was subjected to five freeze−thaw cycles, followed by passing the sample 10 times through a 100 nm polycarbonate membrane using the Mini-extruder (Avanti Lipids). To prepare vesicle-loaded gels, an NaOA vesicle solution of given concentration was combined with a warm (50 °C) solution of gelatin, with the overall pH adjusted to 8.5 using 1 M NaOH. The resulting mixtures were cooled to room temperature to form the vesicle-loaded gels. Further details are discussed in the Results section.

Dynamic Light Scattering (DLS). DLS was used to characterize the sizes of vesicles in solution. A Photocor-FC light scattering instrument with a 5 mW laser source at 633 nm was used at a scattering angle of 90°. A logarithmic correlator was used to measure the intensity autocorrelation function. The hydrodynamic size of the vesicles was extracted from the data using the Stokes−Einstein equation.

SANS. SANS measurements were made on the NG-3 (30 m) beamline at the National Institutes of Standards and

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Technology (NIST) in Gaithersburg, MD. Samples were prepared in D_2O and studied at 25 °C in 2 mm quartz cells. The scattering spectra were corrected and placed on an absolute scale using calibration standards provided by NIST. Data are shown for the absolute intensity I vs the scattering vector $q = (4\pi/\lambda) \sin(\theta/2)$, where λ is the wavelength of incident neutrons and θ is the scattering angle.

Controlled Release Experiments. NaOA vesicles were combined with 15 mM calcein dye. To separate unencapsulated dye, the vesicle−dye mixture was passed through a Sephadex G-50 size-exclusion chromatography (SEC) column. The vesicle fraction (with encapsulated dye) was collected and used in preparing the vesicle-loaded gels. To examine the release of dye, 4 g of aqueous buffer solution was added above 6 g of gel in the headspace of the containing vials. The concentration of calcein in the buffer was measured by UV−vis spectrometry (Cary Bio 50) as a function of time. Release experiments were also done with a control gel (no vesicles). The dye concentration in the control gel was kept identical to that in the vesicle-loaded gel. To determine the latter, 2 mL of the vesicle fraction from SEC was combined with 50 μL of 10% Triton-X100 detergent, thereby disrupting the dye-loaded vesicles and releasing the dye into the bulk solution. The dye absorbance (and thereby concentration) could then be measured accurately by UV−vis, and this concentration was used in preparing the control gel.

3. Results and Discussion

Creation of Vesicle-Loaded Gelatin Gels. We first describe the creation of vesicle-loaded gelatin gels by blending NaOA vesicles and gelatin. We will also address the stability of the vesicles within the gels. As mentioned in the Introduction, NaOA vesicles are made by adjusting the pH of an NaOA solution. The variation in NaOA solution structure as a function of pH is depicted in Figure 1, which is an equilibrium titration curve. To obtain this data, increasing amounts of 1 M HCl were added to a micellar solution of NaOA at pH 10, and the samples were studied by visual observations and light scattering. Phase assignments were done in accordance with our observations, and these are fully in accord with the phase diagram reported previously for oleate solutions by Cistola et al.[16] Photographs of selected samples corresponding to different regions in the phase diagram are shown in Figure 1.

Let us consider the data starting from the origin. At high pH (9.5 or above), NaOA forms solutions of spherical micelles, and these samples are colorless and transparent, as shown by the photograph. As concentrated HCl is added, the solution pH drops from about 9.5 to 7.0, a range that spans the pK_a of NaOA (which is 8.3). Samples in this pH range (interval BC on the plot) are homogeneous and have a strong bluish color, as shown by the photograph. These samples are vesicle solutions and the bluish hue is a manifestation of light scattering from the vesicles (Tyndall effect). With further decrease in pH, the NaOA samples become unstable and inhomogeneous due to the formation of an oil phase (undissociated NaOA). The low-pH samples are turbid, milky emulsions of the oil and water phases, as seen from the photograph.

The aspect to note from Figure 1 is the continuous and reversible transition from NaOA micelles at high pH (> 9.5) to NaOA vesicles at moderate pH (~8.3). The mechanism for this transition (which occurs for all long-chain fatty acids) has been discussed in a number of studies, and the central factor is believed to be the change in the degree of ionization

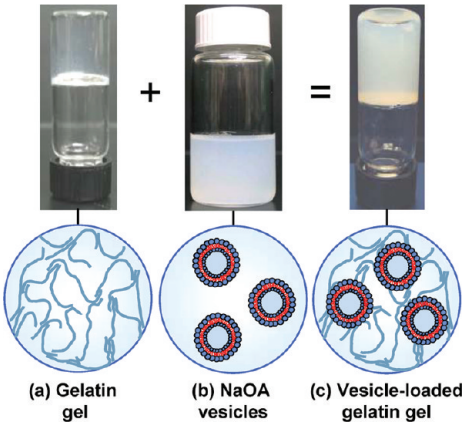

Figure 2. Photographs and schematics of (a) gelatin gel, (b) NaOA vesicles, and (c) gelatin gel loaded with NaOA vesicles. The gelatin gel is a 3-D network of gelatin chains, with chain segments connected into triple helices at the cross-link points. When vesicles of diameter ~100 nm are entrapped in the gelatin gel, the initially colorless gel assumes a bluish hue due to light scattering from the vesicles.

of the fatty acid.[15,16] At high pH, the carboxylate groups on NaOA are fully ionized, and the head groups of the amphiphile thus bear a strong negative charge. The repulsions of these head groups lead to the formation of spherical micelles. Because the micelle size is only around 5 nm, the solutions scatter light weakly and therefore appear transparent. When the pH is lowered to a value close to the pK_a, approximately half the NaOA groups are no longer ionized. The charge may then be considered to be shared by two adjacent fatty acid molecules, one ionized and the other un-ionized: i.e., the molecules are effectively paired into dimers.[15,16] The net amphiphile geometry then becomes conducive to formation of vesicles rather than micelles. The vesicles typically range from 50 to 150 nm in diameter (much larger than the micelles), and therefore the solutions scatter light strongly, leading to the bluish color.

Having ascertained the pH-sensitive nature of NaOA vesicles, we now proceed to discuss their encapsulation within gelatin gels (Figure 2). For this purpose, we first made a 1 wt % NaOA solution at a pH of 8.3 and extruded the vesicles through 100 nm pores to finally obtain vesicles of an average diameter around 100 nm. We then made a solution of 10 wt % gelatin in DI water and adjusted its pH to 8.3. Both the gelatin and NaOA solutions were warmed to 50 °C and then mixed in a 1:1 ratio by weight. As is well-known, gelatin undergoes a sol-to-gel transition when cooled below 35 °C.[5] Upon cooling to room temperature, we obtained a bluish gelatin gel, as shown by the photograph in Figure 2c, with an overall concentration of 5 wt % gelatin and 0.5 wt % NaOA vesicles. Note that gelatin gels not containing vesicles are colorless (Figure 2a), so the bluish color of the gel in Figure 2c is a visual indication that vesicles are indeed intact in the gel. This is further discussed below. Figure 2 depicts the microstructure of the vesicle−gel: it consists of vesicles entrapped in a 3-dimensional network of gelatin chains. The cross-links in the gelatin gel are known to be triple-helical domains where three adjacent chains are linked together.[5]

How can we be sure that the vesicles are intact in the gel? One should note that stable encapsulation of vesicles in gels has already been demonstrated in a number of earlier studies.[9−14] In the present case, we cannot do a DLS experiment with the vesicle-loaded gel itself because

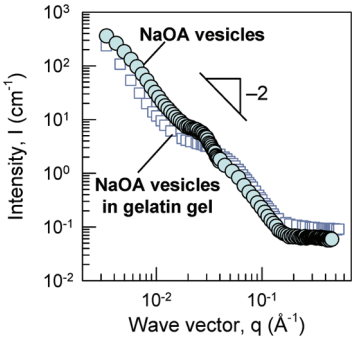

Figure 3. SANS data at 25 °C for 0.5% NaOA vesicles at pH 8.3 (circles) and a 5% gelatin gel loaded with 0.5% NaOA vesicles (squares). In both cases, the intensity I follows a slope of -2 at moderate q, which is characteristic of scattering from vesicles.

the Stokes−Einstein formulation applies only for vesicles in a liquid medium. However, we can put the thermoreversibility of gelatin gels to use here. Specifically, we can heat the gelatin gel until it melts (to ca. 45 °C) and becomes a sol and then check for the vesicle size in the sol by DLS. This can be compared with the DLS results on an NaOA vesicle solution at 45 °C. For 0.5% NaOA vesicles in a 5% gelatin sol at 45 °C, we measured a mean radius of 46 ± 3 nm from DLS. In comparison, for 0.5% NaOA vesicles at the same temperature with no gelatin added, we obtained a similar mean radius of 44 ± 1 nm. These results point to the presence of intact NaOA vesicles in gelatin gels. Incidentally, both the vesicle−gel and its corresponding sol retain the bluish hue of the original vesicle solution.

In addition to DLS, we have also conducted small-angle neutron scattering (SANS) experiments at room temperature on both NaOA vesicle solutions and gelatin gels containing NaOA vesicles. Figure 3 shows a plot of the scattered intensity I vs wave vector q for two samples: 0.5% NaOA vesicles and the corresponding vesicle gel made with 5% gelatin. In both cases, the data follow a slope of -2 at low to moderate q, which is a qualitative indication for the presence of bilayered structures (vesicles).[23] By plotting the above data on cross-sectional Guinier plots (not shown),[24] we can extract the thickness of the vesicle bilayer, which is found to be 3.2 ± 0.2 nm in both cases. Further modeling of the SANS data to compare vesicle sizes is beyond the scope of this paper because, for the vesicle−gel, one would need to account for the contribution from the gelatin network to the scattering intensity. In any case, the SANS data are clearly consistent with the presence of vesicles inside the gel. It is also worth mentioning that in addition to NaOA vesicles, we have also been able to encapsulate phospholipid-based giant unilamellar vesicles (GUVs) in gelatin gels. These vesicles have diameters exceeding 10 μm, and so their presence in the gel is easily confirmed using phase-contrast and fluorescence microscopy (data not shown).

Inducing Vesicle−Micelle Transitions within Gels. The above data confirm that NaOA vesicles can be entrapped within gels. The next question is whether these vesicles still retain their pH-responsive properties. To test the pH response of the vesicle−gels, we initially did the following experiment. We placed a 5% gelatin gel loaded with 0.5% NaOA vesicles in a vial and filled the headspace above the gel with a pH 10 buffer solution (Figure 4). Initially, the entire gel had the bluish color characteristic of vesicles. Within 1 h, we could observe that a portion of the gel closest to the buffer solution had cleared up—it was no longer bluish. As time progressed, the clear front advanced through the gel and by about 9 h, almost the entire volume of the gel had become clear. We also did a control experiment with a pH 8.3 buffer instead of a pH 10 buffer—in this case, no perceptible visual changes were observed in the gel over more than 48 h. These results imply that in the case of the pH 10 buffer the diffusion of the buffer into the gel disrupts the vesicles and transforms them into micelles. Because of the weaker light scattering of micelles, the micellized region of the gel shows up as practically clear. Incidentally, the photographs in Figure 4 were taken after removing the buffer solution from the vial headspace—this was necessary to clearly visualize the gel in the vial. Also, the vial is shown inverted in all the photographs—this is to indicate that the gelatin gel retains its integrity and mechanical strength during the pH-induced transition (there is no change in gel volume either).

The above result is reproducible and can be replicated in other geometries (see below). More quantitative results were gathered from a second experiment in the same vial geometry and are shown in the right-hand panel of Figure 4. In this case, the vesicle−gel was loaded in a cylindrical vial of diameter 7 mm and up to a height of 19 mm. The remaining volume of the vial was filled with pH 10 buffer. The setup was monitored by a camera over the duration of the experiment. From photographs at different instants of time, we were able to monitor the movement of the micelle−vesicle front (i.e., the interface between the micelle-rich and vesicle-rich regions) within the gel. The data (Figure 4) follow the classic diffusive scaling with $\sqrt{t}$.[25−27] In other words, the progression of the front is apparently limited by the rate of diffusion of buffer into the gel; in comparison, the vesicle-to-micelle transition occurs much faster.

We can then model the time-dependent position of the vesicle−micelle interface $h(t)$ by the 1-dimensional diffusion equation:[26]

$$h(t) = h_0 - \sqrt{2Dt} \qquad (1)$$

where D is the diffusivity of the buffer and h_0 is the position at $t = 0$ (which equals the total height of the gel). By fitting this equation to the data in Figure 4, we obtain a diffusivity D of 4.2×10^{-5} cm^2/s for the buffer (hydroxide) ions from the external bath into the gelatin gel. This value is quite comparable to the bulk diffusivity of hydroxide ions in water (5.6×10^{-5} cm^2/s).[28] In other words, the ions diffuse in the gel much like they would in water; this is not surprising since the hydrated size of the ions should be much smaller than the mesh size of the gel. The latter value is estimated from the literature to be about 5 nm for a 5% gelatin gel.[29,30]

We have seen from Figure 3 that it is possible to create a cylindrical gel with different microstructures over different regions. For example, half of the cylinder could contain

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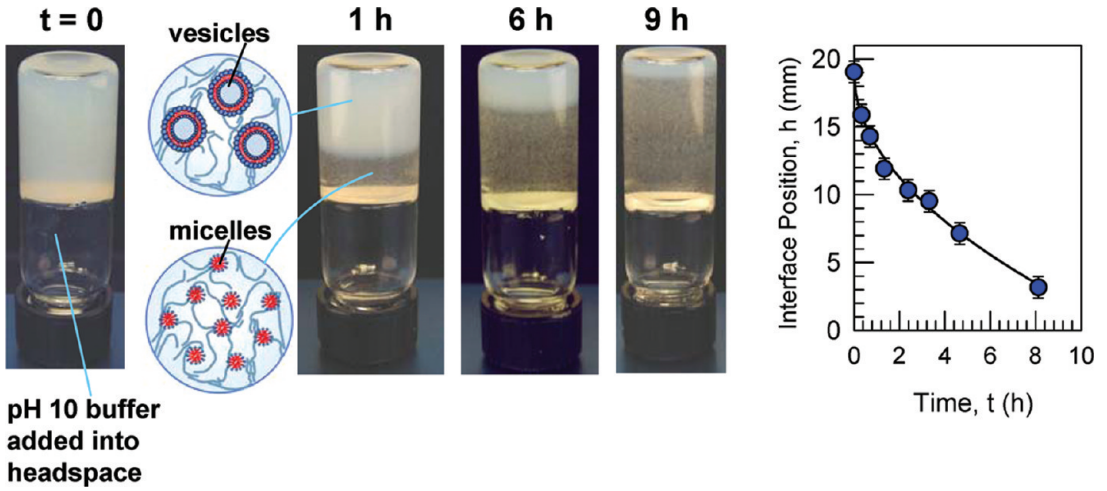

Figure 4. Movement of micellar front within a vesicle gel due to diffusion of pH 10 buffer. The buffer is introduced into the headspace above the gel at time zero. As the buffer diffuses into the gel, NaOA vesicles in the gel are converted into NaOA micelles, as indicated by the change in a portion of the gel from bluish to colorless. As time progresses, the micellar front travels deeper into the gel, indicating that more of the vesicles are converted into micelles. After 9 h, the micelle region covers most of the gel. The plot on the right shows the interface position (measured from the top of the vial) as a function of time. The line through the data is a fit to eq 1.

vesicles and the other half micelles. If the gel is removed from the buffer solution and stored separately, this "pattern" is retained for several days. Similar patterning can also be done with gels of other geometries. For example, we made a vesicle-loaded gel (5% gelatin, 0.5% vesicles) into a spherical ball, 41 mm in diameter. This ball was then immersed in a bath of pH 10 buffer. As the vesicle−micelle front moved radially inward, the vesicles in the outer shell were transformed into micelles whereas the core remained intact. This is evident from Figure 5 (top panel) where we see the clear micellar shell surrounding the bluish vesicle core. With increasing time, the shell becomes thicker, and eventually the entire gel contains only micelles. Thus, we can easily create core−shell patterns with a vesicle core of given size surrounded by a micellar shell.

Another patterning experiment was carried out with the above vesicle−gel in a Petri dish of diameter 90 mm and with the gel thickness being ~10 mm. We then stuck six drinking straws loosely into the gel at several locations. The straws were then filled with pH 10 buffer. As the buffer diffused into the gel, the regions surrounding the straws became clear, indicating that the vesicles in those regions had been converted into micelles. This is shown in Figure 5, bottom panel. The clear regions are roughly circular in cross section, and as time progresses, the circles grow radially outward from the straw positions. Eventually, the clear regions merge with one another, and at this stage most of the vesicles in the gel have been transformed into micelles. Variations of the above experiments can be used to create more complex patterns. As mentioned in the Introduction, an ability to create gels containing pockets of vesicles at precise locations within the bulk structure could have applications in areas such as tissue engineering.

One comment needs to be made regarding the reversibility of the transition, i.e., back from micelles to vesicles. While a vesicle to micelle transition can be readily induced by high pH buffer, the vesicles *cannot* be subsequently re-formed in the gel if it is brought into contact with a low-pH buffer. The reason has to do with the mesh size of the gel, which was estimated above to be ~5 nm. Vesicles of NaOA are thus large enough to be trapped within the gel mesh, but spherical micelles of NaOA, which have a size around 4−5 nm, can "leak" out of the gel and into the external buffer, thereby depleting the NaOA in the interior of the gel. Moreover, for vesicles to be re-formed within the gel matrix, several micelles will need to approach and fuse: again, the mesh is too dense to facilitate such fusion. To re-form vesicles, one would thus have to melt the gel and then combine it with NaOA at the appropriate pH.

Controlled Release from Vesicle−Gels. Finally, we study the controlled release of a dye (calcein) from vesicle-loaded gels, and we examine whether the release kinetics are influenced by pH. First, Figure 6 compares the release of dye from a control gel (no vesicles) and from a vesicle-loaded gel, both containing the same amount of dye. In this experiment, the pH of both the gel and the external solution is at 8.3. In the case of the vesicle−gel, the dye is encapsulated in the vesicles before the vesicles are embedded in the gel (i.e., most of the dye is *inside* the vesicles at time zero). Figure 7 shows that the vesicle−gel releases dye at a slower rate than the control gel. The slower kinetics are evidently due to the additional transport barrier presented by the vesicle bilayers, which the dye molecules must first traverse before releasing into the gel and thereafter into the external solution. Similar results have been found in other studies.[9−14]

Figure 6 also shows the effect of Triton X-100 detergent on the release profiles. This detergent is known to disrupt vesicle bilayers and thereby convert vesicles into micelles.[14] We added Triton to the external solution at the 10 h mark. This had no significant effect on dye release from the control gel, but the release curve from the vesicle−gel shows a sharp increase in slope. Evidently, the Triton molecules diffuse into the vesicle−gel and disrupt the vesicles, causing the encapsulated dye to spill out into the external gel and thus exit the gel at a faster rate; similar results have been reported previously.[14] Eventually, the vesicle−gel release profile catches up with that from the control gel (4 h after Triton addition). The overlap of the two curves confirms that the two gels contain approximately the same overall amount of dye. The Triton experiment is also an indirect proof for the existence of intact vesicles in the vesicle−gel. In addition, the experiment shows how the release rate from a vesicle−gel can

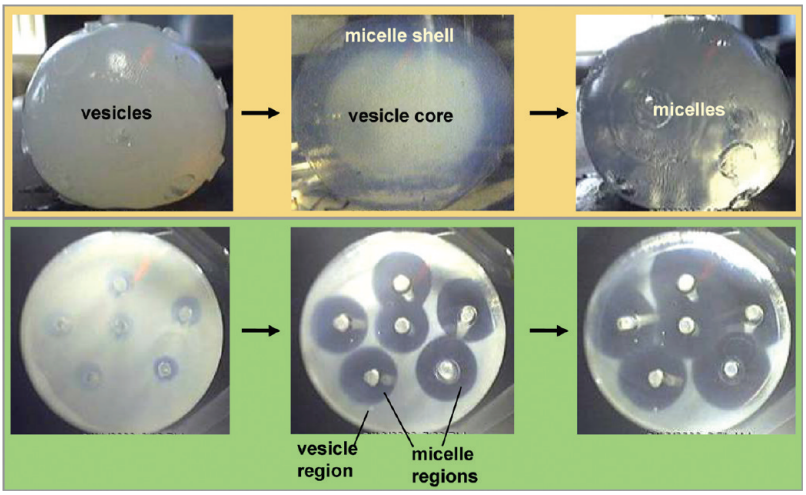

Figure 5. Patterning of vesicle gels by inducing localized vesicle to micelle transitions. Top panel: core−shell structure with a vesicle-rich core surrounded by a micelle-rich shell. This is accomplished by immersing a spherical gel (41 mm diameter) in a pH 10 buffer. The photographs correspond to increasing incubation times in this buffer solution. Bottom panel: gel in a Petri dish with micelle-rich regions created at discrete points. This was done by sticking straws into the gel and subsequently filling the straws with the pH 10 buffer. The photographs again correspond to increasing times and show the expansion of the micelle-rich regions as the buffer diffuses into the gel.

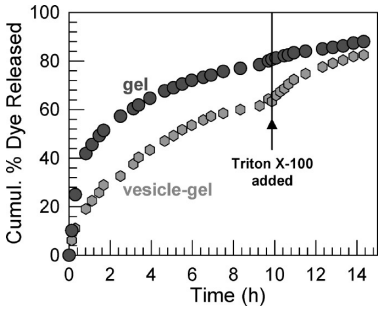

Figure 6. Release profiles of calcein dye from a gelatin gel and from a vesicle-loaded gelatin gel. In the case of the vesicle−gel, the dye was encapsulated within the vesicles, which were then embedded within the gel. The data show a slower release of dye from the vesicle−gel relative to the control gelatin gel. After 10 h, Triton X-100 detergent was added to the external solution for both samples (this point is marked by the arrow). The detergent diffused in and disrupted the vesicles, which lead to an increase in the rate of dye release.

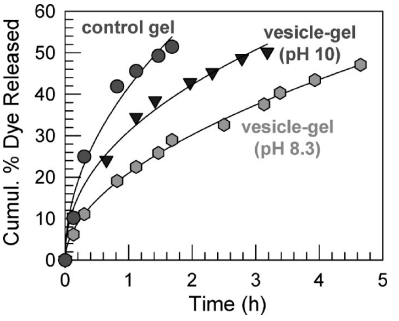

Figure 7. Tunable calcein release from a vesicle-loaded gel based on the pH of the external solution. The control is a gelatin gel surrounded by a pH 8.3 buffer, and in this case (circles), the dye is released rapidly. The same gel loaded with vesicles releases dye much more gradually at pH 8.3 (hexagons): in this case, the vesicles are intact, and the vesicle bilayer thus presents a transport resistance. On the other hand, if the same vesicle-loaded gel is placed in contact with pH 10 buffer, the dye release is more rapid (triangles): in this case, the high pH converts the vesicles into micelles, thereby eliminating the transport resistance due to the vesicle bilayers. All the data are fit to eq 2, and the fit parameters are shown in Table 1.

be tuned by addition of certain molecules to the external solution.

We now discuss the effect of external pH on the calcein release profiles. The results are shown in Figure 7, where data from three experiments are displayed. Two of these are identical to those in Figure 6, namely a control gel (no vesicles) and a vesicle-loaded gel. For these two cases, both the gel and the external solution are at a pH of 8.3. Comparing the results, again the vesicle−gel releases dye much more slowly than the control gel, consistent with the data in Figure 6. The third case is the vesicle−gel placed in contact with a pH 10 buffer at time zero: in this case, the release rate is *higher* compared to the same gel at pH 8.3. This result agrees with our expectations and is similar to the effect of Triton in Figure 6. We expect the pH 10 buffer to diffuse into the vesicle−gel and transform the vesicles into micelles, as shown earlier by the moving front in Figure 3. In turn, the dye encapsulated in the vesicles will be released into the gel matrix, and these molecules would no longer have to contend with the vesicle bilayer as a transport barrier. This explains the net faster release of dye molecules out of the gel. In effect, the dye release kinetics for the pH 10 case gradually begins to resemble that of the control gel (i.e., a gel with no vesicles). Figure 7 thus conceptually demonstrates the use of pH as a trigger to accelerate the release rate of dye from our vesicle−gels.

The dye release curves in Figure 7 can be quantitatively treated using the following equation due to Peppas:[25−27]

$$\frac{M_t}{M_\infty} = kt^n \qquad (2)$$

where M_t/M_∞ is the fractional dye release, k is a rate constant, and n is an exponent characteristic of the transport mechanism. This equation is only valid for short times such that $M_t/M_\infty < 0.6$. Fits of eq 2 to the data are shown in Figure 7, and the fit parameters are shown in Table 1 (in all cases, the quality of the fit was very good, with $R^2 > 0.99$). The exponent n is determined to be 0.49 for the control gel and 0.53 for the vesicle−gel at pH 8.3; both are close to the value of 0.5 expected for Fickian diffusion in a slab

Table 1. Parameters Obtained by Fitting Eq 2 to Dye Release Curves in Figure 7

sample	k	n
(1) gel, no vesicles	0.4	0.49
(2) vesicle gel, pH 8.3	0.2	0.53
(3) vesicle gel, pH 10	0.3	0.45

geometry.[26] For the vesicle−gel at pH 10 the value of n is 0.45. The slight discrepancy in this case is probably because two processes are occurring simultaneously: the vesicles being disrupted into micelles and thus releasing dye into the gel and the free dye in the gel diffusing out into the solution. Note that the rate constant k is highest for the control gel and lowest for the vesicle−gel with intact vesicles (pH 8.3). The acceleration of dye release due to the increase in pH is reflected in a 50% increase in the magnitude of k (from 0.2 at pH 8.3 to 0.3 at pH 10).

4. Conclusions

We have shown that nanoscale vesicles of NaOA can be entrapped within gelatin hydrogels. The resulting vesicle−gel hybrids exhibit the pH-responsive properties of the NaOA vesicles. Specifically, when exposed to a pH 10 buffer, the vesicles within the gel become transformed into micelles. Vesicle disruption can be done in a controlled manner at specific locations within a gel. Gels can thus be "patterned" to have vesicle-rich and micelle-rich domains in predetermined arrangements. The utility of entrapping pH-responsive structures within the gel is in the area of controlled release of hydrophilic solutes. We show that the release of calcein dye out of a vesicle−gel into the external solution is accelerated when the solution pH is raised to 10. This increase is attributed to a pH-induced vesicle to micelle transition within the gel, which reduces the transport resistance to dye diffusion.

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