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Controlled Hydrogel Formation in the Internal Compartment of Giant Unilamellar Vesicles

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The introduction of poly(ethylene dioxythiophene) (PEDOT)/poly(styrene sulfonate) (PSS) polyelectrolyte into giant unilamellar phospholipid vesicles (GUVs) and cross-linking with Ca^{2+} ions to generate a hydrogel within the internal compartment are reported. The aqueous colloidal suspension of PEDOT with excess PSS was microinjected into the internal compartment of liposomes as well as networks of GUVs and lipid nanotubes. The subsequent introduction of calcium ions as cross-linking agent in order to induce hydrogel formation was achieved by three different methods: vesicle fusion, electroporation, and direct microinjection. Gel formation was probed by coinjection of fluorescent nanoparticles and tracking of Brownian motion. Particle mobility was shown to be distinctly reduced in the gel-filled vesicles. Diffusion constants for the particles were calculated from the projected movement of the particles and compared to particles in reference gels and solutions.

Giant unilamellar vesicles are flexible, spherical microcompartments made from surfactant materials. They are mostly composed of phospholipid membranes and can harbor volumes smaller than 10^{-12} L in an aqueous environment. The fluid character of the membrane gives the vesicles dynamic properties, meaning that introduction of liquid through the membrane can be easily achieved, for example, by electroinjection or vesicle fusion. The nature of the liquid content of each vesicle can therefore be freely chosen, provided that the filling solution does not disrupt, destabilize, or dissolve the membrane.[1]

Micro- and nanofluidic devices based on phospholipid membrane vesicles and interconnecting lipid nanotubes have been developed quite recently, and a number of systematic studies have been conducted (e.g., the controlled generation of the micrometer scale networks, their structure and topology as well as modification with biomacromolecules within the compartments, membranes, and nanotubes[2]).

Previously, it has been shown that, with these vesicles and their artificially created networks of interconnecting nanotubes with a typical diameter of 100 nm, a variety of functional aspects can be studied (e.g., reaction kinetics in confined volumes[3]). Applications as a model for cellular functions[4] and the interaction with functional membrane proteins[5] were demonstrated. The biomimetic properties of the membrane also make the vesicles attractive as vessels for model systems in cellular biology (e.g., to study membrane transport phenomena[6] or exo/endocytosis[7]). In comparison to a living cell, such a model system is, because of its lesser complexity and low sensitivity to external stress, far easier to create, to maintain, and to manipulate during an experiment.

However, the lack of equivalents to cytoplasm and cytoskeleton within their primitive structures renders giant vesicles as model systems for chemical reaction kinetics, migration, or diffusion studies in the context of biological cells only valuable with limitations. Fundamental differences in kinetics in cellular environments are attributed to macromolecular crowding, resulting from hindered diffusion, size exclusion, and limited molecular motion in such systems.[8,9] The effect of macromolecular crowding cannot be studied in such simple vesicular microreactors containing only buffer solution.

A cell-sized membrane compartment featuring macromolecular crowding conditions would represent a novel, useful model for quantitative studies of diffusion-controlled kinetics in comparison to macroscopic chemical reactors. Modification of GUVs and vesicle/nanotube networks with a highly viscous or soft solid medium, which can be used to accurately mimic intracellular environments, is desirable.

Presented here is a basic feasibility study with the focus on the combination of functional polymeric materials with fluid membrane devices, adding internal complexity to a structurally simple vesicular system. The goals of the study were the formation of a polymer-based, dense, and biocompatible hydrogel in single giant unilamellar vesicles and networks of such vesicles with nanotubes and the trapping of coinjected nanoparticles in the gel.

One important rationale for the study is the development of methods for the stabilization of GUVs, aiming at a well-defined internal structure of significantly higher density than that of the buffer solution, practically the most basic feature of a biological cell. A second major point of interest in such a system is the possibility of controlled immobilization of chemical and biological material in the context of artificial cells.[10,11] Both aspects are important prerequisites for the study of cellular kinetics in artificially created microreactors under simulated macromolecular crowding conditions.

An attractive material for sol−gel transition studies is the aqueous suspension of the electrically conducting "synthetic metal" poly(ethylene dioxythiophene) (PEDOT) in a solution

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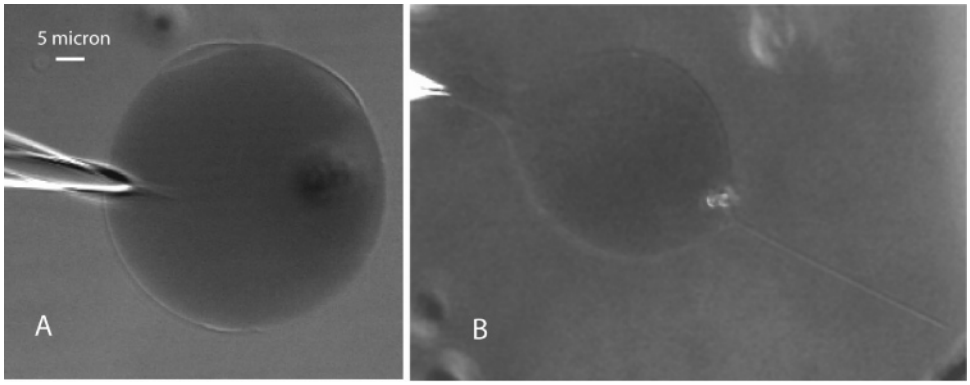

Figure 1. GUV after injection of PEDOT/PSS suspension (A) and after subsequent injection of $CaCl_2$ solution (B). A deformed, gel-filled vesicle in (B) is suspended between the injection needle (left) and the carbon fiber counter electrode (right).

of excess poly(styrene sulfonate) (PSS). This polyelectrolyte has been reported to form hydrogels with high ion conductivity and an electron-conducting polymer backbone[12] and found its first applications in the biosensor area.[13] We established an experimental protocol to directly introduce PEDOT/PSS into giant unilamellar vesicles and to cross-link its main constituent, water-soluble PSS, to form a hydrogel in the internal compartment. Figure 1A shows a 50 μm vesicle microinjected with PEDOT suspension in PSS solution, containing up to 6 wt % of PEDOT with a ratio of PEDOT to PSS of 40:60 and a PEDOT particle size of $<$200 nm.

The injected suspension is only slightly viscous and after adjustment to pH 8 and filtration through a 0.45 μm syringe filter can be easily pressure-injected through a pointed capillary with a 0.5 μm opening. The resulting modified vesicle is stable and appears to maintain its membrane integrity. During our experiments, we did not notice any vesicle content leakage, size change, membrane disintegration, rupture, or even shape deformations other than the ones we artificially induced. However, we did not explicitly test long-term stability and have not yet obtained data on a possible increase in membrane stability.

After the introduction of divalent calcium, magnesium, or tetravalent osmium ions in typically 1 mM solution, gel formation sets in. We selected Ca^{2+} for our protocol, since the effect of calcium ions on the membrane is well-known to be uncritical in the concentration range we used. Hydrogel formation is caused by cross-linking of the polyanionic PSS molecules through binding of metal ions to excess negative charges. PSS is the main constituent of the polyelectrolyte mixture, intended to stabilize positive charges on the polycationic PEDOT backbone, keeping the suspension stable.

Thirty s after metal ion introduction, gel formation has significantly altered the viscosity of the vesicle interior. The ability of the membrane to assume its lowest energy shape is noticeably influenced. Figure 1B shows a vesicle being suspended between the injection needle and the counter electrode, shortly after injection of the PSS-binding solution. The vesicle can be subjected to deformations, which are only slowly reversible. The suspended vesicle features a 10-μm-long protrusion and a second, 45-μm-long tube-like protrusion, which is considerably thicker than nanotubes that normally form in non-gel-filled systems.

A conclusive test of gel formation would be the removal of the membrane by means of a detergent, leaving behind a vesicle-shaped gel structure in the open solution. However, we were not able to reach a gel density high enough to allow removal of the membrane while retaining the shape of the interior compartment. Slow disintegration of the gel structure occurs in the case of treatment with a surfactant such as Triton X. Therefore, immobilization of coinjected material and analysis of the Brownian motion of fluorescently labeled particles was employed to confirm and characterize gel formation.

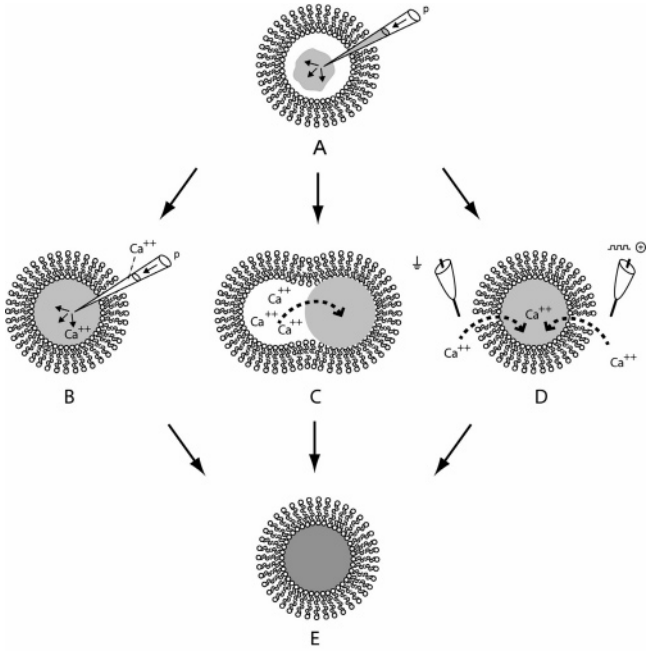

Figure 2. Schematic drawing of hydrogel formation procedure in the internal compartment of GUV. Injection of PEDOT/PSS (A), subsequent injection of Ca^{2+}(B), vesicle fusion (C), or electroporation (D) lead to the hydrogel internalized GUV (E).

Introduction of Ca^{2+} Ion Solution. The material properties of the fluid phospholipid membrane offer at least three different pathways for the introduction of bivalent metal ions through the membrane (Figure 2). After pressure-driven microinjection of the PEDOT/PSS polyelectrolytic suspension (A), either microinjection using a capillary similar to the one used for vesicle creation (B) or vesicle membrane fusion (C) or destabilization of the membrane by an electric field (electroporation) (D) leads to diffusion of ions through the membrane.

All three methods have been employed, achieving hydrogel formation in a GUV of typically 50 μm in diameter, as shown in Figure 3. Best results with highest densities were obtained with the microinjection and electroporation methods. Gel formation upon vesicle fusion is dependent on the size of the Ca^{2+}-containing vesicle. Ca^{2+}-holding containers exceeding 20 μm in diameter did not lead to a dense gel because of the resulting dilution of the PEDOT/PSS suspension.

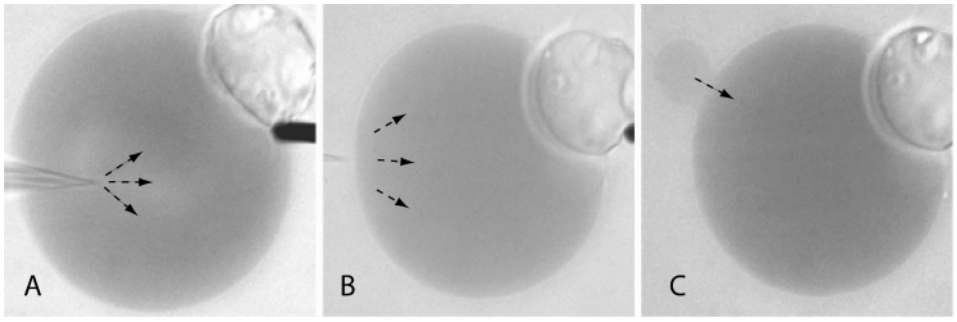

Figure 3. Methods for introduction of metal ions for gel formation into a giant unilamellar vesicle. (A) direct microinjection, (B) electroporation, (C) vesicle fusion. The arrows represent the influx of cross-linking ions, as schematically also depicted in Figure 2. The dark-colored, PEDOT/PSS-filled GUV in the image is connected to a lighter-colored multilamellar vesicle, which acts as the membrane reservoir during the injection.

Network Formation. The formation of networks of nanotubes and containers is achieved using the injection/inflation protocol with subsequent immobilization of the newly created daughter vesicle on the surface (Figure 4A). Network formation was confirmed by addition of a membrane-soluble dye (FM1-48), which fluoresces brightly and highlights membrane areas (Figure 4B). Using microinjection or fusion as a method of introduction of PSS complexing cations, one vesicle at a time can be selectively transformed into a gel-filled GUV. The electroporation procedure can potentially be used to transform the whole network, if multiple electrodes or high field strength in connection with closely spaced vesicles are employed. In addition, diffusion of Ca^{2+} ions through a nanotube is available as a fourth method in nanotube-connected GUV systems.

To demonstrate the possibility of creating complex, stable networks consisting of individual vesicles with different internalized materials, vesicles 1−4 (Figure 4) were microinjected with the polyelectrolyte suspension, while vesicle 5 was filled with buffer. If this vesicle is subsequently injected with, for example, Ca^{2+} ion-enriched buffer, diffusion through the nanotube interconnecting vesicles 1 and 5 initiates the gel formation.

Coinjection of Fluorescent Beads and Controlled Immobilization of the Beads. To establish proof of hydrogel formation and to demonstrate the limiting of free diffusion of coinjected material entrapped in the hydrogel, the movement of individual particles was followed over a period of 30 s, using computer-aided video analysis and spot tracking on a captured movie.

The tracker was locked onto two individual particles simultaneously, recording their movements. Prior to Ca^{2+} injection, the Brownian motion of the fluorescent particles could be observed as largely undisturbed, while in the gel state, the beads were efficiently trapped and displayed reduced visible random movement.

In Figure 5A, the path of two individual particles is displayed (represented as red and blue lines), showing unrestricted Brownian motion. Only very limited individual movement is remaining after gel formation, indicating that the blocking of Brownian motion of coinjected material can be very effectively achieved within this microcompartment/hydrogel system (Figure 5B).

On the basis of the particles' projected movement obtained from the tracking data (Figure 5) and the Einstein−Sutherland relation for Brownian motion,[14] diffusion constants for the particles and viscosities of the media have been calculated. In Table 1, the results for the four tracked particles (taken from Figure 5) are summarized together with the values for particles tracked under identical conditions in bulk gel, in pure buffer, and in a poly(*N*-isopropyl acrylamide) reference gel.[15]

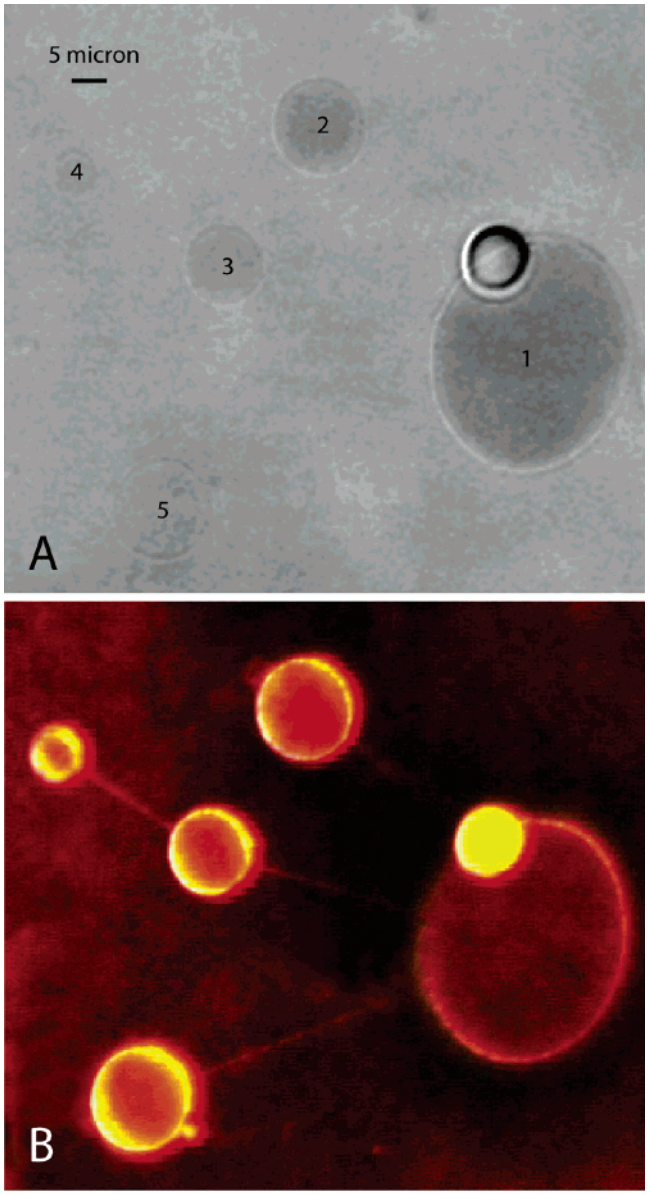

Figure 4. Bright field of a membrane dye-treated vesicle/nanotube network after gel formation. Vesicles 1−4 contain PEDOT/PSS hydrogel; vesicle 5 contains buffer. The vesicles are numbered in the order of creation (A). (B) shows the fluorescence image revealing the connectivity of the containers by nanotubes. The numbering represents the sequence of vesicle formation with injection needle change after step 4.

In comparison, diffusion constants of individual particles are lower by a factor of 2−10 in cross-linked gel than in the non-

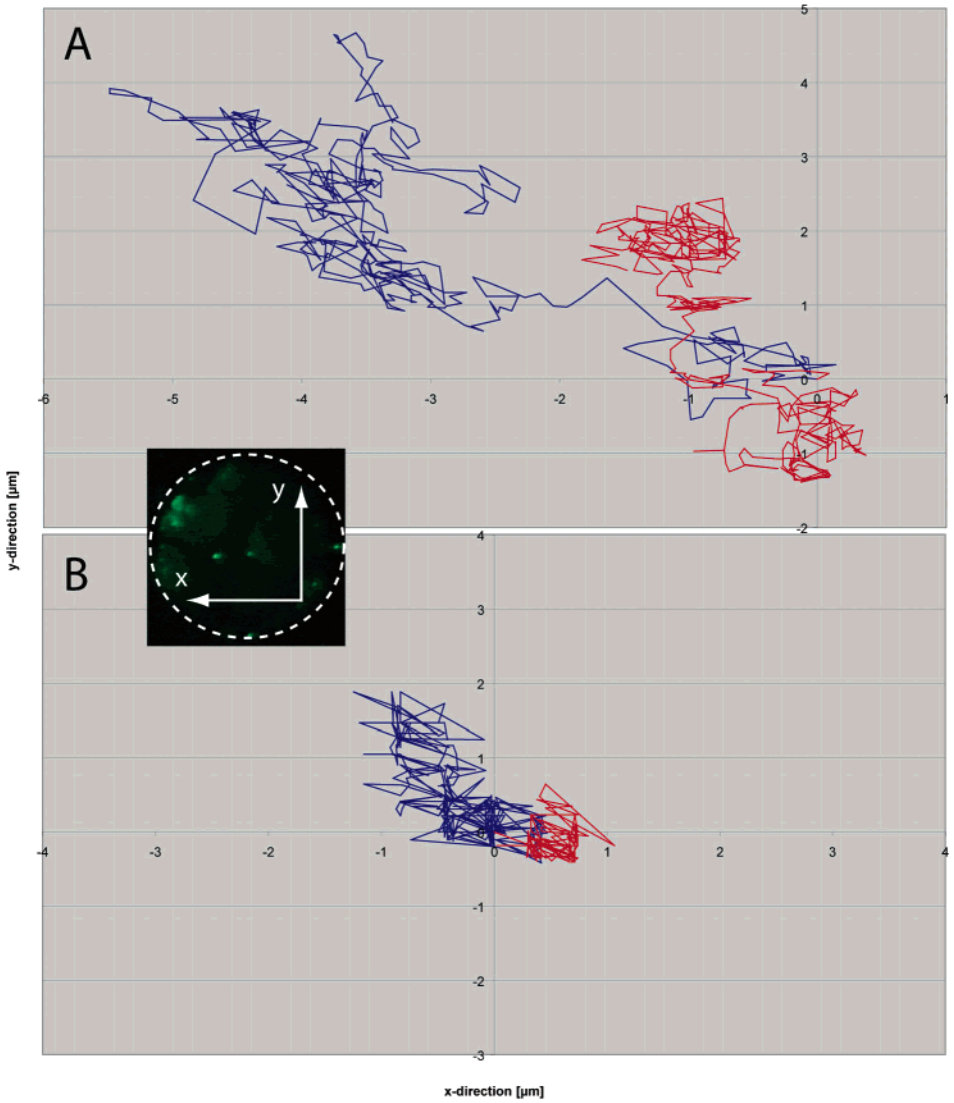

Figure 5. Paths of particle movement in a vesicle. Two individual particles were tracked simultaneously, blue and red in color. (A) Unrestricted Brownian motion of two particles in the unmodified suspension before Ca^{2+} injection. (B) Restricted movement of two different particles after Ca^{2+} microinjection. The inset shows a fluorescence microscopy image of a typical vesicle used in the measurement.

TABLE 1: Calculated Diffusion Constants for 200 nm Fluorescent Latex Beads and Viscosities of the Surrounding Medium[a]

	D_{mean} (m^2/s)	σ	viscosity [kg/(m·s)]
PDOT/PSS gel 1 GUV	1.73×10^{-14}	3.04×10^{-15}	0.123
PDOT/PSS gel 2 GUV	2.26×10^{-14}	5.01×10^{-15}	0.095
PDOT/PSS sol 1 GUV	1.7×10^{-13}	4.68×10^{-14}	0.013
PDOT/PSS sol 2 GUV	5.07×10^{-14}	1.01×10^{-14}	0.042
ref. buffer 1 GUV	5.02×10^{-13}	1.02×10^{-13}	0.004
ref. buffer 2 GUV	6.59×10^{-13}	1.01×10^{-13}	0.003
ref. PDOT/PSS bulk gel	1.44×10^{-14}	8.09×10^{-15}	0.149
ref. PDOT/PSS bulk gel, rediss.	4.6×10^{-13}	9.38×10^{-14}	0.005
ref. PNIPAam gel 1 GUV	2.78×10^{-15}	8.5×10^{-16}	0.770
ref. PNIPAam gel 2 GUV	1.74×10^{-15}	5.67×10^{-16}	1.234
ref. PNIPAam sol 1 GUV	1.27×10^{-14}	3.26×10^{-15}	0.168
ref. PNIPAam sol 2 GUV	2.7×10^{-14}	1.13×10^{-14}	0.079
ref. nonmoving	3.27×10^{-16}	2.03×10^{-16}	6.558

[a] ref. = reference; nonmoving reference = artificially created spot, simulating a totally immobilized particle in order to quantify the intrinsic noise of the tracking software; rediss. = redissolved, bulk produced gel was introduced into excess buffer solution and allowed to dissolve completely. Data processing and calculation details are available as Supporting Information.

cross-linked polyelectrolyte mixture. Bulk gel is slightly denser, and redissolved bulk gel nearly reaches the viscosity of pure buffer solution. A reference PNIPAam gel is almost 10 times more viscous than PEDOT/PSS gel, on the basis of a comparable tracker analysis. It has to be noted that the PNIPAam sol, from which the sol−gel transition was initiated, was already considerably more viscous than the solution injected into the vesicles here in this study. The artificial spot, introduced as a nonmoving control, has a calculated (pseudo-)diffusion constant which is another order of magnitude below that of a particle in the dense PNIPAam reference gel, practically representing the limit of this particle tracker method.

Concluding Remarks

We have demonstrated a system of membrane-enclosed microcompartments filled with picoliter amounts of PEDOT/PSS polyelectrolyte suspension in physiological buffer, which can be subjected to bivalent metal ions to undergo a sol-to-gel transition, leading to the controlled formation of a hydrogel within the vesicle. No noticeable volume changes of the vesicles during the course of the transition are apparent from our experiments, and the membrane remains undisrupted during and after gel formation. To demonstrate a basic function of the gel-modified vesicle interior, coinjected material (e.g., fluorescent, carboxylic-acid-modified 200 nm latex beads) could be trapped

in the gel, efficiently slowing Brownian motion. The sol−gel transition and with it the trapping of coinjected beads is induced here by chemical cross-linking, which stands in contrast to the temperature-controlled, reversible phase transition of a similarly internalized poly(*N*-isopropyl acrylamide) gel, which we reported earlier.[15] Diffusion constants and gel viscosity are accessible through an analysis of the two-dimensional projection of the particles' Brownian motion. Since the internalized gel in the microcompartments is essentially free of complex bulk movement, vesicular systems might also prove useful for further, more accurate studies of gel properties and behavior.

The system reported here, because of its dense interior structure, mainly represents a useful starting point for the utilization of bilayer-enclosed microcompartments as a simple biological cell model. The reported electron conductivity of the polymeric backbone in the hydrogel was not exploited in this initial study, but is important for the future application of the vesicles or vesicle/nanotube networks in electrochemistry-based biocompatible sensors,[13] owing to the high electrode-to-electrolyte surface ratio (supercapacitor),[16] and redox-driven ion exchange capabilities, which was reported in a similar polyelectrolytic system.[17] These aspects as well as the gel properties in the thin, 100 nm lipid nanotubes, which interconnect the vesicles, are subjects of further, more extensive studies, having in mind the development of micrometer-sized membrane-enclosed biosensors.

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Supporting Information Available: Procedures for preparation and immobilization of giant unilamellar vesicles, microinjection procedure, detailed description of microscopy and video data analysis methods used, Matlab function for the calculation of diffusion constants from video tracker data. This material is available free of charge via the Internet at http://pubs.acs.org.

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