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Composite gel-filled giant vesicles: Membrane homogeneity and mechanical properties

Clément C. Campillo [a], André P. Schroder [b], Carlos M. Marques [b], Brigitte Pépin-Donat [a,*]

[a] Structure et Propriétés d'Architectures Moléculaires, UMR 5819(CEA-CNRS-UJF)/INAC/CEA-Grenoble; 17, rue des Martyrs, 38054 Grenoble Cedex 9, France
[b] Institut Charles Sadron, Univ. Louis Pasteur, CNRS - UPR 22; 23, rue du Loess, BP 84047, 67034 Strasbourg Cedex 2, France

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ABSTRACT

We further investigate the properties of composite Poly(NIPAM) (poly(*N*-isopropylacrylamide)) gel-filled giant vesicles, focusing here on i) the homogeneity of the membrane, ii) its coupling to the inner gel under strong suction pressures, and iii) the relation between the final elastic modulus of the vesicles and the amount of crosslinker in the pre-gel medium. We show that whereas the photo-polymerization process induces a decrease of the membrane homogeneity at the micrometer size range, the membrane still remains strongly coupled to the internal gel network. The vesicles studied here display average moduli in the range [0.5–25] kPa, confirming their potential as biomimetic mechanical systems.

1. Introduction

In the living realm phospholipids bilayers build the membranes of cells and cellular organelles [1], they host also proteins responsible for functions as diverse as anchoring the cytoskeleton, providing coating protection against the body immune response or opening ionic channels for osmotic compensation. Giant Unilamellar Vesicles (GUVs) [2] consist in a self-closed phospholipid bilayer. They can provide simple models to understand cell membrane properties such as transport, fusion or mechanical resistance because they exhibit sizes similar to that of cells (0.5 to 100 μm) and the basic properties of biological membranes. In order to be able to model also the cell mechanical properties, one has to modify the viscosity and elasticity of the vesicle interior. Recently, several groups have reported the formation of composite vesicles encapsulating polymer solutions [3,4] or polymer gels [5–8]. We have introduced a method to form giant vesicles encapsulating a viscous solution of poly(NIPAM) or a gel of the same polymer (respectively sol-GUVs and gel-GUVs) [9,10]. Because of the thermo-responsive properties of poly(NIPAM), both sol-GUVs and gel-GUVs volume transition can be triggered by the temperature [11] and hold promise as biomaterials for temperature-controlled drug delivery or as "artificial cells" able to propel in thermal gradients. In a previous paper we presented preliminary measurements of the elastic modulus of composite vesicles containing 3% of crosslinker [9]; we also revealed a strong interaction between the gel and the membrane. A recent study of membrane nanotubes extrusion from gel-GUVs disclosed the presence of structural heterogeneities in the membrane [12]. In the present paper, we further characterize those gel-filled giant vesicles, focusing on i) the membrane homogeneity, ii) the membrane cohesion with the inner gel, and iii) the quantitative study of the overall elastic modulus of the composite vesicles as a function of the crosslinker concentration. The results are obtained by associating fluorescence microscopy and micropipet aspiration. We show in particular that the elastic modulus of the composite vesicles can be varied over more than one decade by controlling the initial cross-linker concentration.

2. Materials and methods

2.1. Chemicals for preparation of composite Giant Unilamellar Vesicles (GUVs)

Phospholipids used in this work are 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC, 99%, Sigma-Aldrich) and fluorescent 1-oleoyl-2-[12-[(nitro-2-1,3-benzoxadiazol-4-yl)amino]-dodecanoyl]-sn-glycero-3-phosphocholine (NBDPC, 99%, Avanti Polar Lipids). The gel precursor mixture (pre-gel) encapsulated by the vesicles was prepared from the monomer *N*-isopropyl-acrylamide (NIPAM, Acros Organics), the cross-linker *N,N′*-methylene-bis-acrylamide (MBA, Sigma-Aldrich) and photo-initiator of radical polymerization 2,2-diethoxyacetophenone (DEAP,

* Corresponding author.
E-mail addresses: schroder@ics.u-strasbg.fr (A.P. Schroder), brigitte.pepin-donat@cea.fr (B. Pépin-Donat).

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Acros Organics). All compounds were used as received. Chloroform (Sigma-Aldrich), methanol (Normapur), sucrose (99%, Sigma-Aldrich), glucose (99%, Sigma-Aldrich) and all other reagents are of analytical grade.

2.2. Preparation of the precursor mixtures

The pre-gel aqueous solution was made from sucrose (0.1 M), NIPAM (0.3 M), DEAP (4.85 mM) and various MBA concentrations: 9, 18 and 27 mM corresponding respectively to 3%, 6% and 9% of the monomer molar fraction. Oxygen dissolved in the pre-gel solution was removed by bubbling dry Argon for 30 min before the experiments.

The pre-sol solutions were made from a similar mixture without the crosslinker.

2.3. Fabrication of Giant Unilamellar Vesicles enclosing pre-gel or pre-sol mixtures

GUVs enclosing all the compounds required for gel (resp. sol) polymerization are prepared using the standard electroformation technique [13]. 20 µL of 1 mg/mL lipid in chloroform solution was spread on two metallic (ITO) glass plates, and then allowed to dry in primary vacuum for 3 h. The two plates were assembled by a Sigillum wax (Vitrex, Copenhagen, Denmark) spacer to form a closed chamber filled with the pre-gel (resp. sol) solution. A 10 Hz alternative electric field is applied according to the following protocol: 20 min at 0.2 V, 1 h at 0.4 V, 3 h at 0.6 V and finally 1 h at 4 Hz, 0.6 V. The GUVs are then removed from the growth chamber and kept in the dark in an Eppendorf vial filled with argon.

2.4. Formation of the gel and sol-filled Giant Unilamellar Vesicles

The preparation of the gel and sol-filled Giant Unilamellar Vesicles (gel-GUVs and sol-GUVs) follows closely the preparation method previously described [10]. All the steps leading to the gel (resp. sol) formation are performed under controlled Argon atmosphere inside a glove box that contains also one UV-B irradiation lamp (Sunlight-Erythemal, Harvard Apparatus). Irradiation is performed through a band-pass filter (320 nm–400 nm). The solution holders are three glass containers resting on ice packs and covered with a quartz window. The band-pass filter prevents phospholipids damage. The ice pack controls the solutions temperature during photo-polymerization and the quartz window allows transmission of the UV radiation. The measured optical power density below the quartz window is 19 µW/cm^2 as measured by a silicon photodiode LM2 with an X1000 attenuator and coupled to a Field Master GS from Coherent. In the case of the gel-GUVs, the three containers are filled with identical solutions that combine i) 1 mL of the so-called dilution solution, similar to the pre-gel mixture but without the crosslinker and with saccharose replaced by glucose and ii) 50 µL of the pre-gel GUVs solution described above. In the case of the sol-GUVs, the three containers are filled with 1 mL of the pre-sol GUVs solution. All the samples were irradiated for 5 min, removed from the glass containers and stored at 4 °C.

2.5. Optical microscopy

We used an inverted microscope Nikon TE 200, with a X40 DIC objective. A mercury lamp provided the illumination for fluorescence experiments. A fluorescence block with filters EX 450–490 nm/BA 520 nm, and a Dichroic Mirror 505 nm was used. Pictures were recorded with a digital camera (Diagnostic Instruments NDIAG 1800) onto the hard disk of a Personal Computer, with a pixel depth of eight bits. In order to prevent quenching of the fluorescent probe, the vesicles were first localized and observed in bright field conditions, under low illumination. An initial series of experiments on fluorescent GUVs were performed to determine the optimal experimental conditions for quantitative fluorescence measurements. We have optimized the sets of values of i) diaphragm aperture, ii) neutral grey filter value, and iii) camera gain and exposure time, so as to minimize photobleaching and to maximize the signal to noise ratio. The determined set of the experimental parameters was then kept constant in all measurements, except for the exposure time of the camera that was chosen such as to avoid signal saturation. Fluorescence heterogeneities on the membrane were quantified by using a homemade software that analyses the maximum intensity along the azimuthal direction.

2.6. Micropipet aspiration setup

Glass capillaries (Clark Capillaries, GC 100-10, 1 mm OD, 0.58 mm ID, without filament) were used to make the pipets. Each capillary is washed with ethanol and stretched with a pipet puller (Sutter Instruments P97). The pulled pipet has a conical shape with a tip diameter of the order of 1 µm. It is first coarsely cut with a tweezer, under the microscope, to a diameter of circa 3 µm and eventually forged to the desired diameter with a homemade microforge. The pipet is hosted by an injection holder (Narishige HI-7) connected to a homemade hydraulic watertight set up that allows to apply and quantify (pressure gauge Honeywell ref. 26PCCFA6D±1 atm) a suction pressure in the range 10–10^5 Pa with a precision of 10 Pa. The pipette holder can be manipulated with micrometric precision.

2.7. The mechanics of micropipet gel suction

The geometry of the experimental setup for micropipet suction is sketched in Fig. 1. A cylinder with internal radius R_p and external radius R_e is brought into contact to a semi-infinite elastic medium. When a suction pressure is applied, the elastic body interface is deformed by the applied stresses. One denotes by L the maximum deformation of the interface inside the micropipet. The deformation amplitudes outside or under the pipet are much smaller than L, their exact shape will depend on the boundary conditions that apply. In the simple limit case where the elastic medium can be described as an elastic homogenous body of Young modulus E. Théret et al. [14,15] have shown that for small deformations the applied pressure ΔP induces a deformation L that can be expressed as:

$$L/R_p = (3\Phi/2\pi E)\Delta P \quad (1)$$

where Φ is a constant that depends on boundary conditions (full-adhesive or non-adhesive contact between the pipet tip and the gel surface, see reference [14]) and on the relative magnitudes of R_p and R_e. In most situations of practical interest $\Phi = 2.1$. The Young modulus

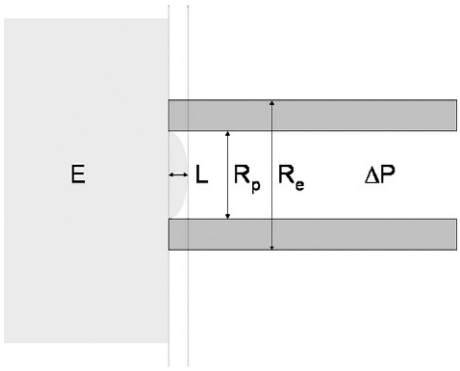

Fig. 1. Aspiration of a half-space of elastic modulus E in a micropipet of internal radius R_p with a aspiration pressure ΔP, the part of the gel aspirated in the pipet has the length L.

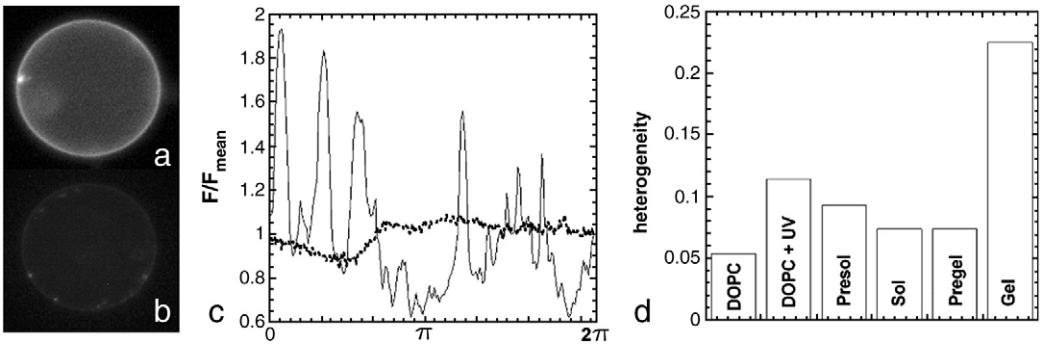

Fig. 2. (a) DOPC GUV observed in fluorescence microscopy (b) Gel-GUV observed in fluorescence microscopy (c) Azimuthal plots of the fluorescence measured along the vesicle contour at the level of the equator. The fluorescence is normalized by its mean value. The dotted curve corresponds to a DOPC GUV filled with a 0.1 M saccharose solution and the full line to a gel-GUV (d) Heterogeneity values of the fluorescence, defined as the ratio of the standard deviation over the mean value for DOPC GUVs filled with 0.1 M saccharose, DOPC GUVs filled with 0.1 M saccharose after UV illumination, pre-sol GUVs, sol-GUVs, pre-gel GUVs and gel-GUVs.

can thus be evaluated from the slope of the plots $\Delta P(L/R_p)$ in the small deformation limit where $L \ll R_p$.

3. Results and discussion

3.1. Membrane homogeneity as seen from fluorescence microscopy

In our previous study [9] we have already shown the possibility to fluorescently label the composite vesicles' membrane. In this part we determine how the various steps involved in the sol-GUVs and gel-GUVs preparation modify the membrane integrity. To do so we quantify the heterogeneity of the composite vesicles' membrane.

Fig. 2(a) displays a DOPC vesicle whilst a gel-GUV is shown in Fig. 2(b). The comparison of the azimuthal plots of fluorescence intensity for a typical DOPC vesicle and a typical gel-GUV is reported in Fig. 2(c). The figure shows deviations from the mean much larger for the gel-GUV. The ratio of the standard deviation over the mean value provides a quantitative measure of the displayed heterogeneities. Fig. 2(d) displays the latter quantity, averaged over several vesicles, for the different types of GUVs studied in this work. A significant increase in fluorescence heterogeneity is clearly revealed for gel-GUVs as compared to all the other systems. This shows that heterogeneities in the fluorophore distribution are associated to UV effects in the presence of NIPAM and crosslinker. Nanotube extrusion experiments allowed us to show that in the case of gel-GUVs, contrary to what happens in bare DOPC or sol-GUVs, only a fraction of lipids is allowed to flow in the tube [12]. This result could not be interpreted by the adhesion of the membrane to the gel by few strong point-like adhesion sites. We assumed local homogeneous adsorption of the poly(NIPAM) chains of the network on the lipid bilayer leading to a "mosaic" structure of the membrane. The "hot spots" of fluorescence observed here might be related to these "patches" of lipids of lower mobility.

3.2. Structure of the gel and sol-filled vesicles as probed by micropipet suction

In order to further probe the structure of the composite vesicles we applied localized mechanical stresses by the micropipet suction method. For the sol vesicles we test the mechanical resistance of the membrane to rupture. For the gel vesicles we test both the mechanical coupling of the membrane to the internal network and the effective elastic modulus of the composite spheres.

3.3. Membrane behaviour under mechanical stress

The mechanical resistance of the membrane to an applied stress was tested for different vesicles using the micropipet aspiration technique. The micropipet suction pressure ΔP corresponds to a membrane tension σ given by the Laplace expression $\sigma = \Delta P R_p / 2(1 - R_p/R_v)^{-1}$, where R_p and R_v are respectively the internal micropipet and vesicle radii [16]. For DOPC GUVs we found that the membrane is disrupted, on average, at tensions larger than 0.7 +/− 0.4 mN/m. For the sol-GUVs that disruption occurs at the lower average tension value 0.3 +/− 0.07 mN/m, thus revealing no enhanced resistance of the membrane induced by the presence of poly(NIPAM).

The membrane integrity of the gel-GUVs was also probed by micropipet suction. Fig. 3(a) displays a bright field image of a gel-vesicle sucked into the pipet and Fig. 3(b) the related fluorescence picture. The applied pressure is in this case $\Delta P = 1000$ Pa. As the figures

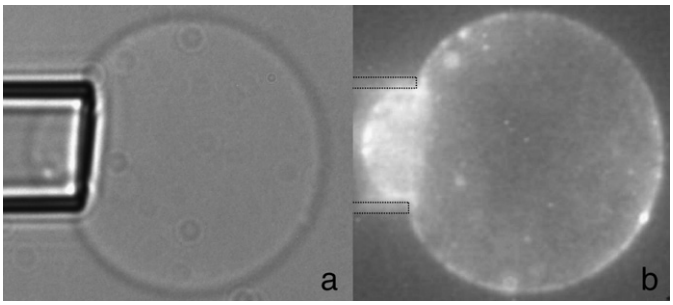

Fig. 3. Transmission (a) and fluorescence (b) observation of a gel-GUV aspirated in a micropipet with $\Delta P = 1000$ Pa, revealing the colocalization of the gel and the membrane.

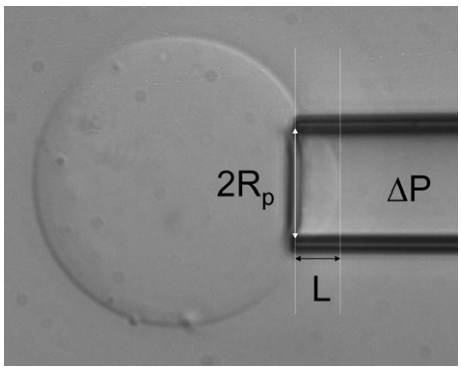

Fig. 4. Aspiration of a gel-GUV in a micropipet of radius R_p with an aspiration pressure ΔP, the part of the gel-GUV aspirated in the pipet has the length L.

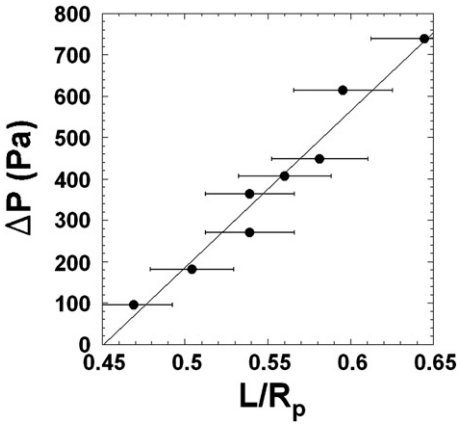

Fig. 5. Projected length L normalized by R_p versus aspiration pressure ΔP during a typical gel-GUV aspiration experiment.

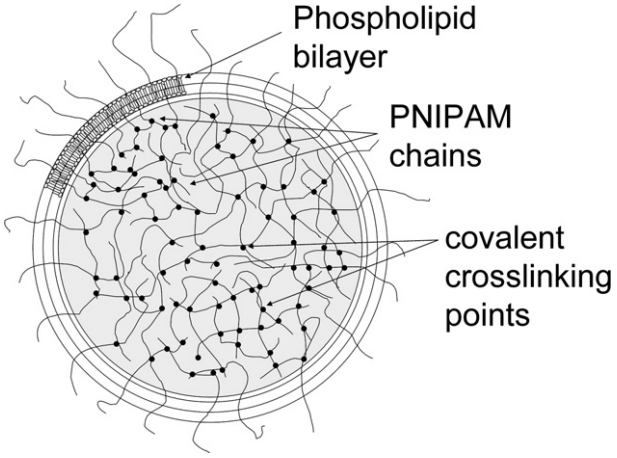

Fig. 7. Scheme of the composite structure of poly(NIPAM) gel-GUVs.

clearly show, the membrane remains attached to the gel and is not macroscopically ruptured by the applied stress, sub-optical damage might go undetected by the visualization technique. For the non-composite DOPC GUVs the suction pressure ΔP would have induced a tension σ=15 mN/m, well above the disruption tension that we have measured for DOPC and sol-GUVs. This indicates that the two membrane leaflets are likely to be strongly coupled to the internal polymer network. Although the exact origin of the coupling between the inner poly(NIPAM) gel and the membrane remains to be determined, we already know that this coupling cannot be attributed to covalent bonds formed between the chains and the unsaturated bond of DOPC during the polymerization. Actually we have shown that fully saturated DMPC membranes exhibit the same strong coupling with poly(NIPAM) gel (data not shown). This result shows that this coupling is not completely due to the presence of covalent bonds between the NIPAM network and the lipid bilayer. This conclusion is further confirmed by membrane nanotube extrusion study [12], which reveals that adhesion of the membrane to the gel is not due to few strong point-like adhesion sites.

3.4. Effective mechanical properties of gel-GUVs

The strong coupling of the phospholipid membrane to the internal polymer gel ensures the continuity of the strain field from the membrane surface down to the interior of the vesicle. From the point of view of the suction experiments, the composite vesicle appears as an effective elastic body of modulus E. In a typical experiment the micropipet is brought into contact with the vesicle surface and a small pressure increment is applied. This leads to a new equilibrium shape of the gel surface and thus to a new value of L, as sketched in Fig. 1 and actually shown for a composite vesicle in Fig. 4. The elastic modulus is extracted from the linear regime of small deformations, by using Eq. (1). Numerical simulations of similar experiments show that the strain field induced by the micropipet aspiration is localized near the micropipet tip, ensuring that, even when the aspirated gel-GUV is not very large compared to the micropipet, a linear elasticity analysis is still relevant [17].

Fig. 5 shows representative results for a suction experiment. Particular care is taken to work in the small strain regime as required for comparison with the linear theoretical analysis presented above. This requires at least avoiding strain singularities at the micropipet tip such as those associated with the presence of a gel cylindrical section. Further conformity with the linear approximation is obtained by ensuring that the relative deformation L/R_p is smaller than unity.

Fig. 6 presents values of the elastic moduli E for gel-GUVs prepared with three different MBA (crosslinker) concentrations: 3, 6 and 9%. Each of the E values is extracted from experiments on a different vesicle. In spite of a significant dispersion of the order of 50%, spanning the range [0.5–25] kPa, the results show a consistent increase of the elastic modulus with crosslink concentration; the structure of the internal medium of gel-GUVs is homogeneous and can be experimentally tuned by the crosslink ratio. A tentative scheme of a gel-GUV structure is depicted in Fig. 7.

The values of E reported here and values of E for macroscopic samples of similar gels are of the same order of magnitude (6 to 12 kPa for crosslink ratio of 0.5 to 1.3%, for example [18]).

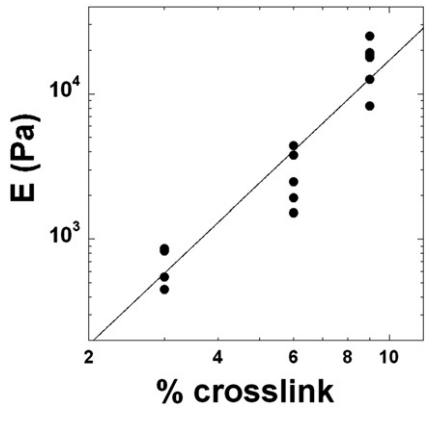

Fig. 6. Elastic moduli E measured on gel-GUVs from samples prepared with different crosslink ratios (% molar).

4. Conclusions

We have studied the structure and mechanical properties of poly (NIPAM) gel-filled giant vesicles by a combination of microscopy and micromanipulation techniques. We have shown that the composite vesicles withstand stress and behave like a continuous elastic material when submitted to pressures high enough to allow for gel elastic moduli determination. Contrary to DOPC and sol-filled vesicles, the membrane of the gel-GUVs is not disrupted by the application of suction pressures as high as 10^5 Pa. The resistance of the gel-filled vesicles to such pressures reveals a strong coupling between the membrane and the inner gel. We were able to vary the mechanical susceptibility of the composite vesicle in a controlled manner by modifying the initial crosslink ratio. In the range of the studied crosslink ratios, composite vesicles exhibit elastic moduli ranging between 0.5 kPa and 25 kPa similar to that of living cells (3 to 5 kPa for fibroblasts [19] or macrophages [20], 5 to 50 kPa for

platelets [21] or 10 to 200 kPa for cardiocytes [22]), showing that such vesicles are good candidate systems for mimicking cell mechanical behaviour.

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