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TITLE:

Obtention of Giant Unilamellar Hybrid Vesicles by Electroformation and Measurement of their Mechanical Properties by Micropipette Aspiration

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KEYWORDS:

giant unilamellar vesicles, micropipette, copolymer, lipid, hybrid polymer/lipid vesicle, membrane properties, area compressibility modulus, bending modulus, electroformation, bilayer

SUMMARY:

The goal of the protocol is to reliably measure membrane mechanical properties of giant vesicles by micropipette aspiration.

ABSTRACT:

Giant vesicles obtained from phospholipids and copolymers can be exploited in different applications: controlled and targeted drug delivery, biomolecular recognition within biosensors for diagnosis, functional membranes for artificial cells, and development of bioinspired micro/nano-reactors. In all of these applications, the characterization of their membrane properties is of fundamental importance. Among existing characterization techniques, micropipette aspiration, pioneered by E. Evans, allows the measurement of mechanical properties of the membrane such as area compressibility modulus, bending modulus and lysis stress and strain. Here, we present all the methodologies and detailed procedures to obtain giant vesicles from the thin film of a lipid or copolymer (or both), the manufacturing and surface treatment of micropipettes, and the aspiration procedure leading to the measurement of all the parameters previously mentioned.

INTRODUCTION:

Giant vesicles obtained from phospholipids (liposomes) have been widely used since the 1970s as the basic cell membrane model[1]. In the late 1990s, vesicular morphologies obtained from the self-assembly of copolymers, named polymersomes in reference to their lipid analogs[2,3], rapidly appeared as an interesting alternative to liposomes that possess weak mechanical stability and poor modular chemical functionality. However, their cell biomimetic character is rather limited

compared to liposomes since the latter are composed of phospholipids, the main component of the cell membrane. Furthermore, their low membrane permeability can be an issue in some applications like drug delivery where controlled diffusion of species through the membrane is required. Recently, the association of phospholipids with block copolymers to design hybrid polymer-lipid vesicles and membranes has been the subject of an increasing number of studies[4,5]. The main idea is to design entities that synergistically combine the benefits of each component (bio-functionality and permeability of lipid bilayers with the mechanical stability and chemical versatility of polymer membranes), which can be exploited in different applications: controlled and targeted drug delivery, biomolecular recognition within biosensors for diagnosis, functional membranes for artificial cells, development of bio-inspired micro-/nano-reactors.

Nowadays, different scientific communities (biochemists, chemists, biophysicists, physico-chemists, biologists) have increasing interest in development of a more advanced cell membrane model. Here, our goal is to present, as detailed as possible, existing methodologies (electroformation, micropipette aspiration) to obtain and characterize the mechanical properties of giant vesicles and the recent "advanced" cell membrane models that are hybrid polymer lipid giant vesicles[4,5].

The purpose of these methods is to obtain reliable measurement of the area compressibility and bending moduli of the membrane as well as their lysis stress and strain. One of the most common techniques existing to measure bending rigidity of a giant vesicle is fluctuation analysis[6,7], based on direct video microscope observation; but this requires large visible membrane fluctuation, and is not systematically obtained on thick membranes (e.g. polymersomes). Area compressibility modulus can be experimentally determined using the Langmuir Blodgett technique but most often on a monolayer[8]. The micropipette aspiration technique allows the measurement of both moduli on a bilayer forming giant unilamellar vesicle (GUV) in one experiment.

The following method is appropriate for all amphiphilic molecules or macromolecules able to form bilayers and, consequently, vesicles by electroformation. This requires a fluid character of the bilayer at the temperature of electroformation.

PROTOCOL:

1. Fabricating micropipettes

NOTE: Here, micropipettes with an inner diameter ranging from 6 to 12 µm and a taper length around 3-4 mm are necessary. A detailed method of manufacturing micropipette is described in the following.

1.1. Place the borosilicate glass capillary in the drawbar of the puller and fix one of the ends by tightening the knob.

1.2. Carefully slide the glass through the holes at the side of the heater chamber.

1.3. Tighten down the clamping knob at the other end.

1.4. Control the size of the tip and the taper length to achieve the desired specifications. For that, optimize technical parameters such as heating temperature, pull, velocity, delay and pressure. Here is an example of a program used:

Heat: 550 °C
Pull: 10 (Range of the machine: 0-255 in arbitrary units)
Velocity: 30 (Range: 0-255 in arbitrary units)
Delay: 1 (Range: 0-255 in arbitrary units)
Pressure: 500 (Range: 0-999 in arbitrary units)

1.5. Click on **PULL** to execute the events defined by the program. The capillary is then separated into two micropipettes, whose dimensions have to be adjusted using a microforge.

1.6. Insert the micropipette into the metal pipette holder of the microforge (see **Figure 1**). By using 10x objective, adjust the microscope stage and the pipette manipulator (vertical and horizontal movement) until the pipette tip is close to the glass bead surface.

1.7. Press the foot switch to melt the glass bead. Lower the tip and put it in contact with the molten glass bead. Molten glass will flow into the pipette by capillary action. Wait a few seconds until the level of the molten glass reaches a certain height as shown in the insert of the **Figure 1**.

1.8. Stop the heating by removing the pressure on the foot switch, and quickly pull the tip away using the vertical pipette manipulator to cause a sharp break.

1.9. Repeat steps 1.7 and 1.8 until the desired diameter is obtained (6 to 12 µm).

NOTE: To improve the accuracy of the diameter measurement, during the last step, use a 32x objective equipped with a reticle.

2. Coating pipette tips with BSA (**bovine serum albumin**)

2.1. To prepare a 0.1 M solution of glucose containing 1% wt. BSA in pure water.

2.1.1. Weigh 180 mg of glucose powder, place in a 15 mL polypropylene conical tube and complete with 10 mL of pure water.

2.1.2. Add 0.1 g of BSA powder and shake gently using a test tube rotatory mixer until complete dissolution (approximately 4 h).

2.2. Take the solution with a 10 mL disposable Luer syringe fitted with a needle. Once filled, remove the needle and install a 0.22 µm acetate cellulose filter. Fill several polypropylene micro-

centrifuge tubes (1.5 mL) that will be used to immerse the tip.

2.3. Place the capillaries vertically into holders. Lower the holder and immerse the tip into the glucose/BSA solution overnight. The solution should rise about 1 cm high by capillary action.

2.4. Remove the pipette tip from the glucose/BSA solution. Prepare 5 mL of 0.1 M glucose solution (dilute 90 mg of glucose powder in 5 mL of pure water) and filter through a 0.22 µm acetate cellulose filter.

2.5. Fill the pipette with the glucose solution by using a 500 µL glass syringe equipped with a flexible fused silica capillary. Then, remove all the glucose solution by sucking it back and discard it (**Figure 2**). Repeat this step several times to remove the unbounded BSA.

3. Formation of GUVs and GHUVs by electroformation

NOTE: Electroformation is a commonly used technic developed by Angelova[9]. The procedures to obtain an electroformation chamber, deposit a lipid or polymer film (or both for GHUVs (Giant Hybrid Unilamellar Vesicles)) and hydrate the film under an alternative electric field are described in the following. The procedure to collect the GUV obtained is also described.

3.1 Amphiphile, sucrose and glucose solutions preparation

3.1.1. Prepare an amphiphile solution at a concentration of 1 mg/mL. Weigh 10 mg of amphiphile and dissolve in 10 mL of chloroform. Keep the solution in sealed vials to avoid solvent evaporation.

3.1.2. Prepare a stock solution of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (DOPE-Rhod) at 1 mg/mL in chloroform.

3.1.3. Add 10 µL of fluorescent lipid solution to the amphiphile solution. Keep the solutions in sealed vials to avoid solvent evaporation.

3.1.4. Prepare sucrose and glucose solutions at a concentration of 0.1 M. Weigh 342 mg and 180 mg of sucrose and glucose, respectively, and dissolve them in 10 mL of pure water.

3.2. Preparation of the electroformation chamber

NOTE: Different conductive materials can be used to make an electroformation device (e.g., platinum wires[10], stainless needles[11]). The electroformation chamber is composed of two ITO slides separated by an O-ring rubber spacer that has been cut on one side to create an aperture. The slides are connected to a voltage generator via two electric wires (**Figure 3** and **Figure 4A**).

3.2.1. Clean the ITO slides with organic solvent (e.g., chloroform). Identify the conductive surface using an ohmmeter.

3.2.2. Attach the electrical wires on the conductive side using adhesive tape.

3.2.3. Amphiphile solution deposition

3.2.3.1. Dip a capillary in the solution until the level increases by capillary action and collect about 5 µL of the solution.

3.2.3.2. Put the capillary in contact with the center of the ITO glass plate and gently spread the solution. Wait 10 seconds to ensure complete solvent evaporation (**Figure 4A**).

3.2.3.3. Repeat this procedure 3 times for each side.

3.2.4. Add a layer of silicon-free grease on both sides of the opened O-ring spacer. Put it around the area of deposition. Place the conductive face of the second ITO glass plate on the top of the spacer.

3.2.5. Place the electroformation chamber under vacuum for 3 hours to remove any traces of organic solvent.

3.3. Electroformation procedure

3.3.1. Plug the electric wires to the generator.

3.3.2. Use the following settings for the generator:
Alternative sinusoidal tension
Frequency: 10 Hz
Amplitude: 2 $V_{peak\text{-}to\text{-}peak}$

NOTE: Optimum voltage frequency and duration must be found for each system.

3.3.3. Ensure that the voltage is applied before injection of the solution in the chamber.

3.3.4. Inject 1 mL of solution using a syringe with 0.8 mm inner diameter needle to fill the chamber. Remove eventual bubbles.

3.3.5. Let the chamber under the applied voltage/frequency for 75 min (**Figure 4B**).

3.4. GUVs harvest

3.4.1. Switch off the generator.

3.4.2. Using 1 mL syringe with 0.8 mm inner diameter needle, suck a small part of the solution in order to produce an air bubble inside the chamber. Slightly tilt the chamber in order to make

this bubble move inside the chamber. This can help the GUVs to detach from the ITO surface (**Figure 4C**).

3.4.3. Suck all the solution and transfer it into a 1 mL plastic tube.

3.4.4. Remove the wires and clean the ITO slides with organic solvents (toluene then chloroform).

4. Micromanipulation set up

NOTE: The principle of micropipette aspiration is to suck a single vesicle through a glass micropipette by applying a depression. The length of the tongue inside the pipette is measured as a function of the suction pressure. The pipette coating with BSA, described previously, is essential to prevent or minimize any adhesion between vesicles membranes and the pipette. The protocol is illustrated below.

4.1. Pipette and water filled reservoir connection

NOTE: The water filled tank and the micrometer are fixed to a sliding plate. A digital counter with a micrometer head allows a vertical displacement of the device in a range of 0 to 2.5 cm and an accuracy of 1 µm. Displacement along an aluminum optical rail is possible up to 1 meter in length. A silicon tubing connects the reservoir and the capillary holder (**Figure 5A**).

4.1.1. Fill the tank with pure water. Connect a disposable 5 mL syringe to the capillary metal holder *via* silicone tubing and aspirate to create a water flow from the tank to the holder.

4.1.2. Touch the tube slightly to eliminate air bubbles. Simultaneously, raise the water tank to create a positive pressure. The 5 mL syringe is still attached to the holder.

4.1.3. After coating and cleaning steps described previously (see step 2), fill a capillary with glucose solution until a drop forms at the end. Remove the syringe tubing from the metal holder to create a slight water flow at its end.

4.1.4. Turn the capillary upside down and connect the glucose drop with the water flow from the holder. Fix the capillary and the holder by screwing them together.

4.2. Position a pipette

NOTE: During this operation, the water tank is still positioned on the aluminum rail to maintain a positive pressure.

4.2.1. Take the homemade aluminum stage equipped with two glass slides (previously cleaned with ethanol) and glue them with vacuum grease on each side. Install it on the microscope stage and form a meniscus between the two slides by using a 1 mL syringe containing 0.1 M of glucose

as shown in **Figure 5B,5C**.

4.2.2. Place the pipette and its holder on the motor unit of the micromanipulator and tighten down clamping knob.

4.2.3. Use the control panel joystick in coarse mode to lower the micropipette near the glucose meniscus. Adjust the position of the tip to the center of the meniscus using the fine mode.

4.2.4. Hold the tip immersed in glucose for a few minutes to clean its outer and inner surface (as a positive pressure is maintained, the water flow will rinse the inner surface of the pipette to remove uncoated BSA).

4.2.5. Store the position of the tip on the micromanipulator keyboard and withdraw it from the meniscus.

4.2.6. Remove the glucose meniscus and replace it by a fresh one. Suck 2 µL of GUVs in 0.1 M sucrose by using a 20 µL micropipette and put it in the fresh glucose meniscus. Observe in DIC mode (differential interference contrast) the GUVs located at the bottom due to the difference of density between sucrose (mainly inside the GUVs) and glucose (mainly outside the GUVs).

4.2.7. When the vesicles are slightly deflated, reinsert the suction pipette and focus on the tip. Set the height H_0 of the water tank for which pressure is almost 0. For that, take advantage of small vesicles or particles which are naturally present in solution and adjust the water tank height until the motion of these particles is stopped.

4.2.8. At this point, surround the meniscus with mineral oil to prevent water evaporation, see **Figure 5D**.

NOTE: The room temperature should be controlled between 20-22 °C by using air conditioning.

4.3. Micropipette aspiration experiment

4.3.1. Lower the pipette tip (6-12 µm in diameter) and create a small suction (-1 cm) to aspirate a vesicle (15-30 µm in diameter). The membrane of the selected vesicle should slightly fluctuate, and must not present any visible defects (no bud or filament) (**Figure 6**).

4.3.2. Raise the pipette to a higher level to isolate the aspirated vesicle from the other vesicles, by using the micromanipulator and keep this position during the whole experiment.

4.3.3. Pre-stress the vesicle by lowering the water filled tank to approximately -10 cm, then decrease the pressure to return to its initial value (-1 cm). Repeat this step several times to remove membrane excess and small defects from the membrane.

4.3.4. From a height of -0.5 cm defined by the position of the water tank, slowly decrease the

suction pressure with the micrometer to reach a regime in which the membrane fluctuates. Then increase the pressure to clearly visualize a tongue in the tip with a significant projection length (a few microns).

NOTE: The lowest applied pressure (P_0) that allows sucking up the smallest membrane projection length (L_0) will define the reference point α_0 (**Figure 7A**). All points of the curve will be measured according to this reference ($\Delta L = L-L_0$ and $\Delta P = P-P_0$).

4.3.5. Increase the suction pressure with the micrometer in a stepwise manner until reaching 0.5 -0.8 mN/m. At each step, wait 5 s and take a snapshot of the tongue. This procedure at low tension enables determination of the bending modulus.

4.3.6. Keep on increasing the suction pressure from 0.5 mN/m to the rupture tension by adjusting the height of the water filled sliding on the rail (ranging from -2 to -50 cm) (**Figure 7B to 7D**). From this experiment at high tension regime, the area compressibility modulus, lysis tension and lysis strain will be measured.

4.3.7. Stretch about 15-20 vesicles to acquire significant statistics. Each micropipette aspiration experiment takes between 7 and 10 minutes. Perform image analysis using LASAF software for measuring the projection length of the tongue, the diameter of vesicles and the radius of the capillary.

4.4. Measuring bending modulus, area compressibility modulus, lysis tension and lysis strain

4.4.1. To access these parameters, use the formalism established by E. Evans[12]. Calculate the suction pressure applied over the membrane from equation 1:

$$\Delta P = \rho_w g (h - h_0) \quad (1)$$

where g is the gravitational acceleration (9.8 $m \cdot s^{-2}$), ρ_w is the density of the water ($\rho = 1\ g \cdot cm^{-3}$), h is the position of the water tank and h_0 is the initial position where pressure is equal to zero.

4.4.2. Calculate the membrane tension from the Laplace equation:

$$\sigma = \frac{\Delta P}{2} \frac{R_P}{1 - \frac{R_P}{R_V}} \quad (2)$$

where ΔP is the suction pressure on the micropipette, R_p and R_v are the micropipette and vesicle radii (outside the micropipette) respectively. The surface area strain (α) of the membrane is defined as:

$$\alpha = \frac{A - A_0}{A_0} \quad (3)$$

A_0 being the membrane area of the vesicle at the lower suction pressure.

4.4.3 Calculate α from the increase in projection length ΔL of vesicle inside the capillary tip according to Equation 4[12]:

$$\alpha = \frac{1}{2}\left(\frac{R_P}{R_V}\right)^2 \left(1 - \frac{R_P}{R_V}\right)\frac{\Delta L}{R_P} \quad (4)$$

4.4.4 Under a very low tension regime, smoothing of thermal bending undulations dominates the apparent expansion. Plot ln(σ) *vs* α. At low-σ values (typically 0.001–0.5 $mN.m^{-1}$ [13]), this gives a straight line whose slope is linked to the bending modulus, K_b (first term of the equation 5)[14]:

$$\alpha = \frac{kT}{8\pi K_b} ln\left(1 + \frac{A_0\sigma}{24\pi K_b}\right) + \frac{\sigma}{K_a} \quad (5)$$

NOTE: Under high tensions (> 0.5 $mN.m^{-1}$), membrane undulations are completely suppressed and membrane area increases as the result of increased spacing between molecules. In this regime, the second term of equation 5 dominates and give access to area compressibility modulus K_a (**Figure 8** and **Figure 9**).

REPRESENTATIVE RESULTS:

With the protocol aforementioned, we have studied different synthetic giant unilamellar vesicle (GUV), obtained from a phospholipid: 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (POPC), a triblock copolymer: Poly(ethyleneoxide)-*b*-Poly(dimethylsiloxane)-*b*-Poly(ethyleneoxide) (PEO_{12}-*b*-$PDMS_{43}$-*b*-PEO_{12}) synthesized in a previous study[13], and a diblock copolymer Poly(dimethylsiloxane)-*b*-Poly(ethyleneoxide) ($PDMS_{27}$-*b*-PEO_{17}). It has been previously shown by our group that the association of triblock copolymer PEO_8-*b*-$PDMS_{22}$-*b*-PEO_8 with phospholipid POPC leads to a huge decrease of membrane toughness of the resulting GHUVs (Giant Hybrid Unilamellar Vesicles)[15]. The measurement has been repeated for this study and extended to GUVs obtained from diblock copolymer and GHUV obtained from the association of this diblock copolymer and POPC.

The results are illustrated in **Figure 10** and **Table 1**. The area compressibility modulus and lysis strain for POPC are in perfect agreement with literature[16]. The measurement of the bending moduli of the hybrid vesicle has not yet been performed in the laboratory. Typical values are given for the polymersomes obtained. It is worth to mention that the toughness of membrane (E_c) obtained from diblock copolymers is far beyond those obtained with triblock copolymer. More interestingly, with the diblock copolymer it is possible to obtain giant hybrid unilamellar lipid/polymer vesicles that present higher toughness than the liposomes, which is the main interest of such association.

FIGURE AND TABLE LEGENDS:

Figure 1: **Microforge for polishing pipette tips**. The picture shows the different parts of the device: the metal pipette holder (**a**), the glass capillary (**b**), the micromanipulator heater zone (**c**),

the light source (**d**), the 10x, 32x, 40x objectives (**e**), and the microscope oculars (**f**). In the insert, the pipette tip, immersed in a glass bead, is observed through a 32x objective with reticle. The level of the molten glass into the tip has been fixed at an intermediate height (H) after cooling. Pulling the tip away causes a sharp break.

Figure 2: **Experimental set up for coating and filling pipette tips.** (**A**) A 500 µL glass syringe equipped with a flexible silica capillary is mounted to fill the micropipette with a glucose solution. (**B**) Magnification of the capillary lower part. During overnight immersion, the BSA solution level rises by capillary action up to 1 cm in length. The pipette is then filled with glucose by inserting a flexible capillary to remove unadsorbed BSA.

Figure 3: Materials to build an ITO based electroformation device. The device is composed of two glass slides coated on one side with indium tin oxide (**a**). A rubber O-ring has been cut on one side to allow loading of solution inside the chamber and harvest of the GUVs suspension (**b**). The rubber O-ring is used as a spacer to separate the two slides. The slides are connected to the voltage generator via electric wires (**c**) and attached to the surface by adhesive tape (**d**). Silicon-free grease is used to seal the spacer with the slides (**e**). Ohmmeter is used to identify the ITO coated sides and check the conductivity (**f**).

Figure 4: Electroformation set up. (**A**) The amphiphile solution is deposited using a glass capillary on the ITO coated sides of the glass plates. (**B**) After the assembly and the drying step, the chamber is connected to the voltage generator (10 Hz, 2 V) and then filled with sucrose solution. (**C**) After electroformation, an air bubble is created inside the chamber to help the GUVs to detach from the surface.

Figure 5: **Micropipette Aspiration Set Up.** (**A**) Components description: Fluorescence microscope (1), water filled tank and micrometer sliding on an aluminum optical rail (2), silicone tubing conducting water flow from the reservoir to the micropipette (3), micromanipulator control panel (4), motor unit of the micromanipulator which allows X, Y and Z displacement (5), vibration isolation table (6). (**B**) Magnification showing the homemade aluminum stage equipped with two glass slides (7), pipette (8) and pipette holder (9) mounted on the motor unit of the micromanipulator and fixed by clamping knob (10). Note that the pipette tip is immersed at the center of glucose meniscus**.** (**C**) Glass slides glued with vacuum grease on each side allowing the formation of glucose meniscus *(Side view)*. (**D**) Glucose meniscus surrounded by mineral oil to prevent water evaporation *(Top view).*

Figure 6: Image of a GUV under tension using micropipette aspiration. The red channel collecting the fluorescence from the rhodamine tagged lipid and the transmission channel (DIC) have been merged.

Figure 7: Images of a GUV at different suction pressure. (**A**) The lowest applied tension that induces a tongue formation is used as reference to determine the initial tongue length (L_0) and the vesicle radius (R_v). (**B**) Intermediate applied tension value with the associated tongue length

(L). (**C**) Very high applied tension. (**D**) Image just after the membrane rupture where the pipette radius is measured (R_p).

Figure 8: Representative stress-strain plot of GUVs obtained by micropipette aspiration. The same data set has been used to plot ln(σ)=f(α) and σ=f(α). In the low tension regime, ln(σ) varies linearly with α (green linear fit) and gives access to the Bending Modulus (K_c); whereas in the high tension regime, σ varies linearly with α (red linear fit) and gives access to the Area Compressibility Modulus (K_a).

Figure 9: **Representative stress-strain plot of GUV obtained by micropipette aspiration in the stretching regime.** From the experimental curve several mechanical parameters of the GUVs can be determined. The Area Compressibility Modulus (K_a) corresponds to the initial slope and is measured by fitting linearly the curve. The last measured point gives the Lysis Strain (α_L) and the Lysis Stress (σ_L) values. Finally, the Cohesive Density Energy (E_c) can be estimated by integrating the area under the curve (orange area).

Figure 10: **Representative stress-strain plots obtained for Liposome, Polymersome and Hybrid Polymer/Lipid vesicle.** GUVs composed of POPC (red triangles), triblock copolymer (green circles), diblock copolymer (blue squares), triblock-based hybrid (light green circles) and diblock-based hybrid (light blue squares). The curves were obtained by averaging the measurements on at least 15 GUVs for each system.

Table 1: Mechanical parameters determined using micropipette aspiration techniques on GUVs composed of phospholipid, copolymers or both.

DISCUSSION:

The coating of the micropipette is one of the key points to obtain reliable measurements. Adhesion of the vesicle to the micropipette must be prevented, and a coating is commonly used in literature[17-21], with BSA, β-casein or surfasil. Details of the coating procedure are rarely mentioned.

Dissolution of the BSA should be performed for at least 4 hours under agitation in order to achieve good solubilization. Nevertheless, the filtration step is still required to remove any aggregates that may obstruct the micropipette tip. If BSA is not well dissolved, most of it will be removed by filtration, and coating will be ineffective. The ideal concentration and dissolution time are respectively 0.8-1 wt. % and 4 h.

Another critical point is to insure a constant osmotic pressure inside and outside the vesicle during measurement. An increase of glucose concentration due to water evaporation during the experiment can lead to deflation of the vesicle and perturb the measurement (underestimation of K_a, etc.). The deposition of an oil layer is mandatory to prevent this phenomenon (**Figure 3D**). To check the efficiency of the oil layer, a constant aspiration pressure of few $mN \cdot m^{-1}$ can be applied on a vesicle for 5 min, and the length L of the tongue inside the capillary should be constant.

The last critical point is the pre-stress step (section 3.3), rarely mentioned in literature[20]. This step is necessary to remove the buds, the tubes and the excess surface area of the vesicle and get reproducible results from a vesicle to another.

The micropipette aspiration method can be applied on all GUVs, as long as they present a fluid membrane (e.g., $T_{electroformation} > T_m$ of lipids) and possess a bending modulus below 100 kT. In case of a thick and viscous membrane, even in the fluid state, two pipettes can be used to measure the moduli[22]. The micropipette aspiration technique presents a great advantage to give access to several parameters (bending and area compressibility moduli) and this is the only technique available to directly access the area compressibility modulus of a GUV's membrane.

Although this technique has been well known for a long time it is still commonly used by numerous scientific communities (biophysicists, physico-chemists, chemists, etc.). The micropipette aspiration method will continue to be a significant technique in the future, especially to investigate further membrane properties of advanced synthetic cell (e.g. Hybrid Polymer/Lipid Vesicles and protocells).

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DISCLOSURES:
The authors have nothing to disclose.

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Figure

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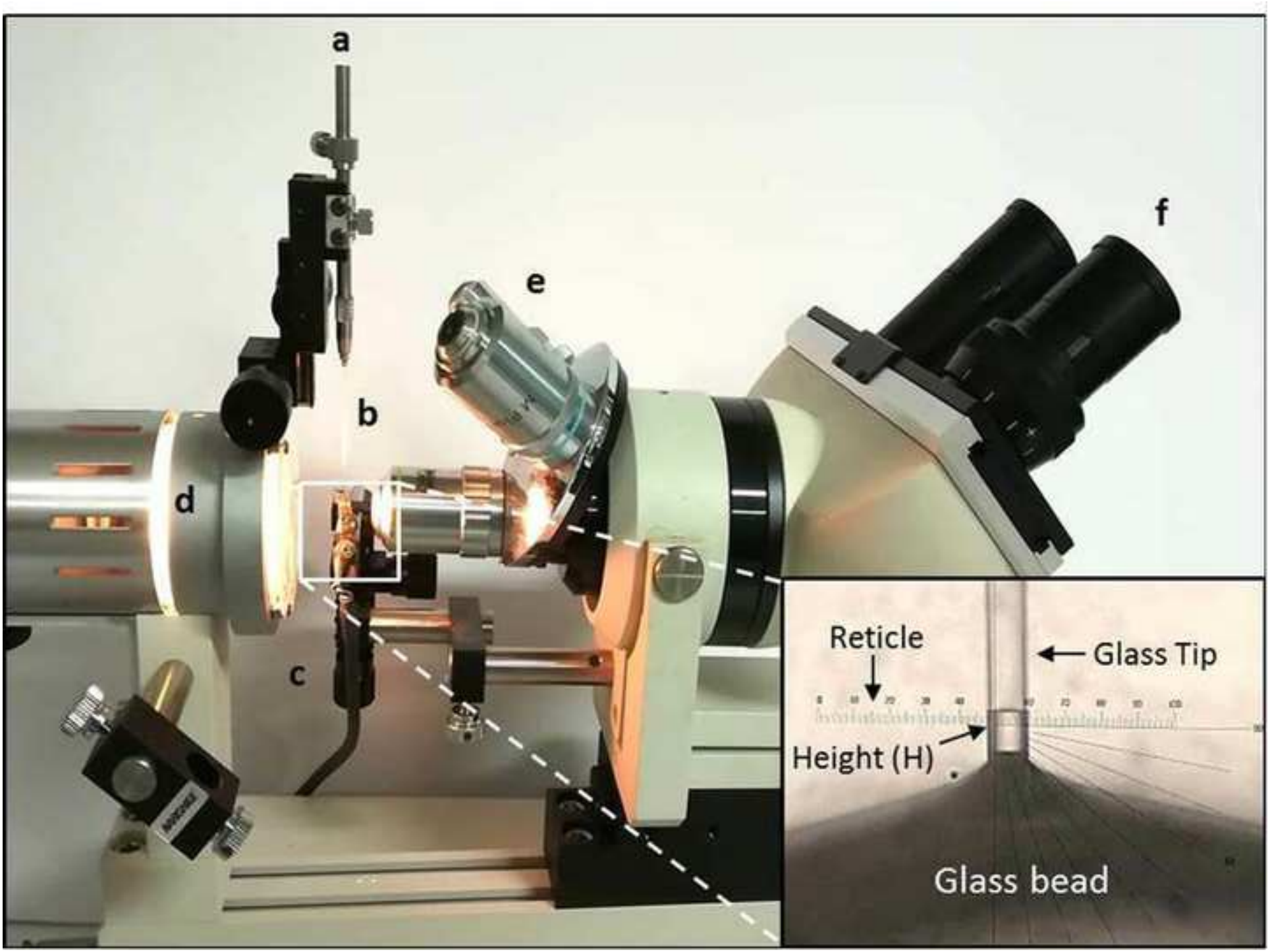

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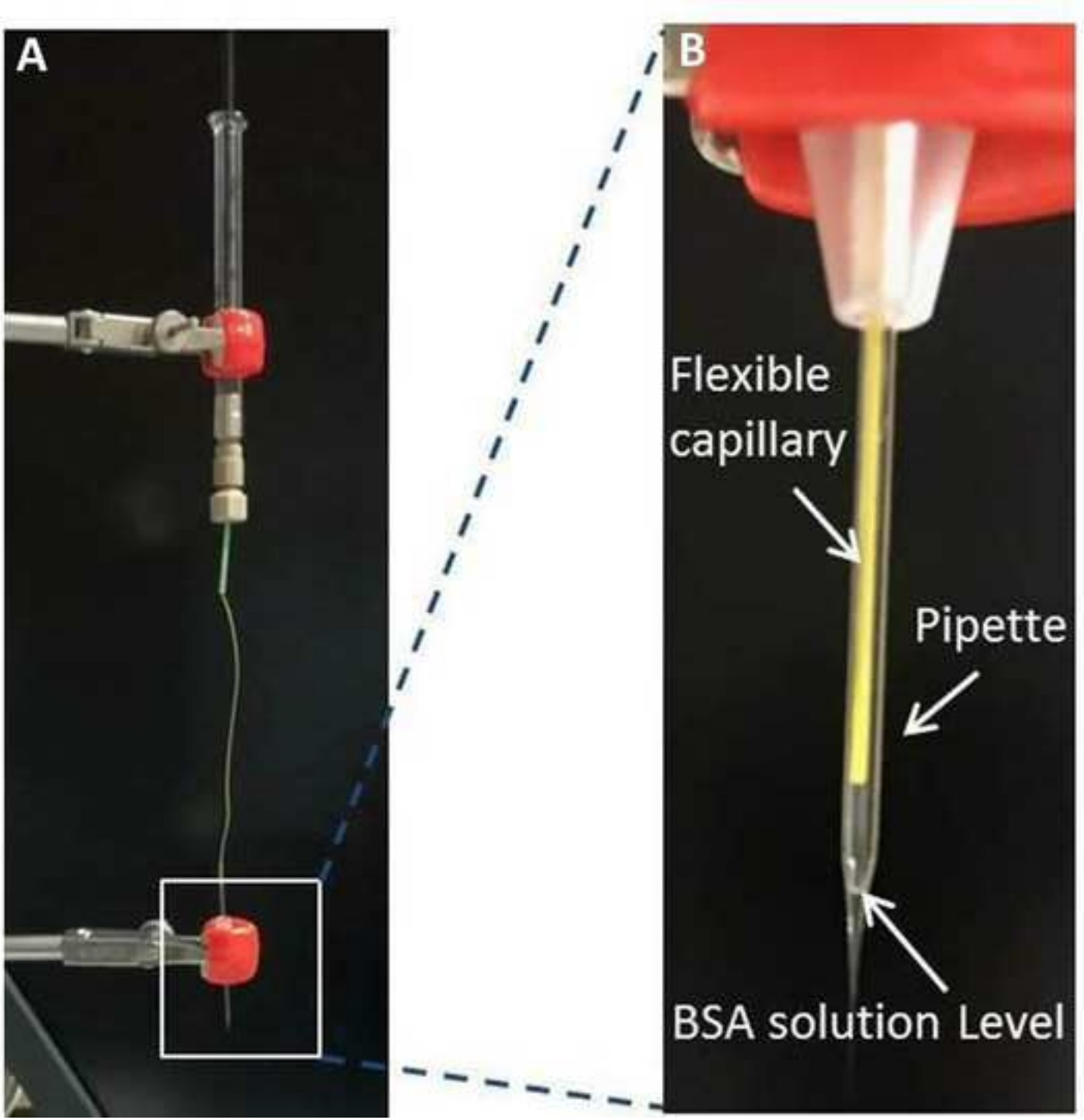

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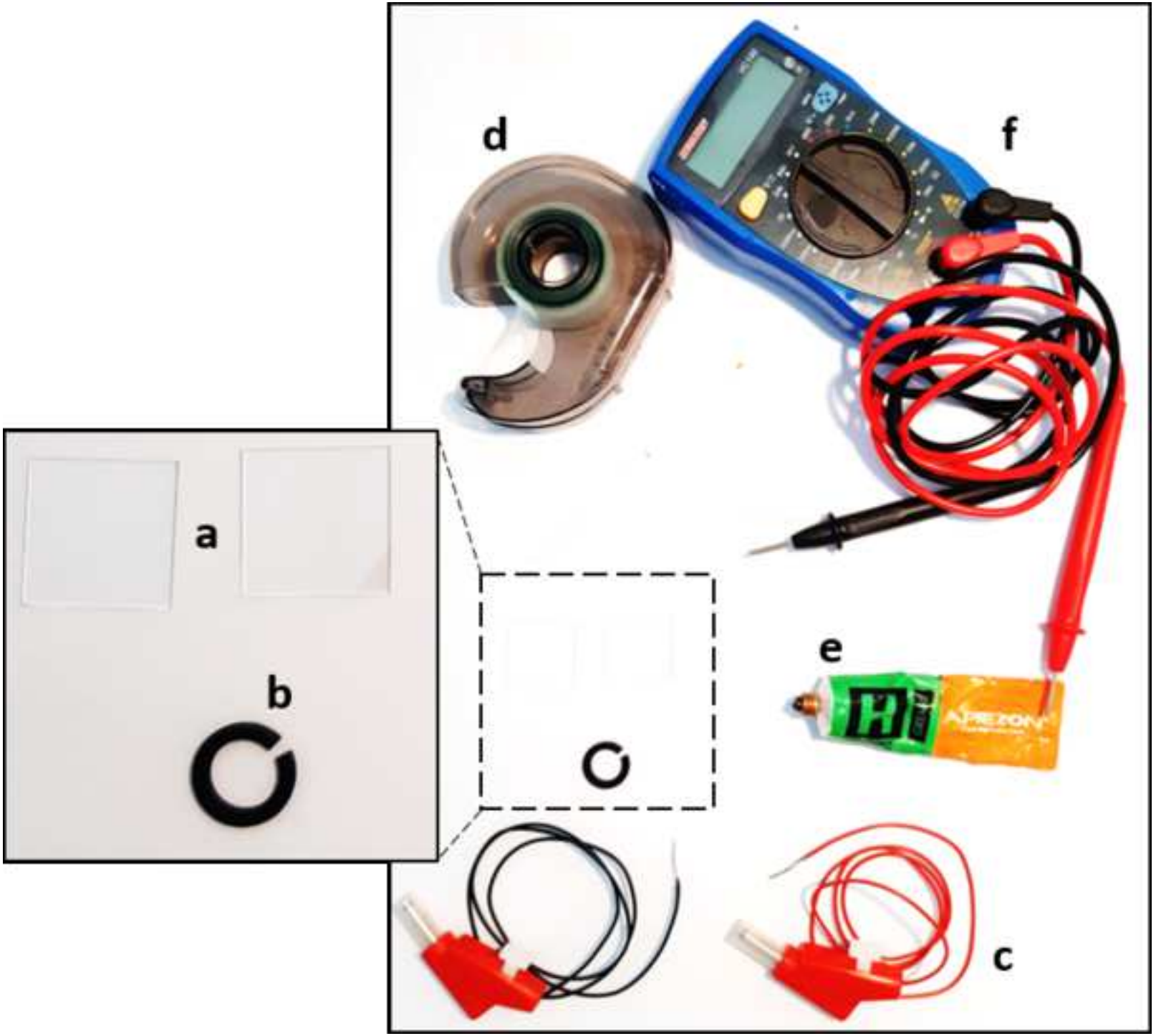

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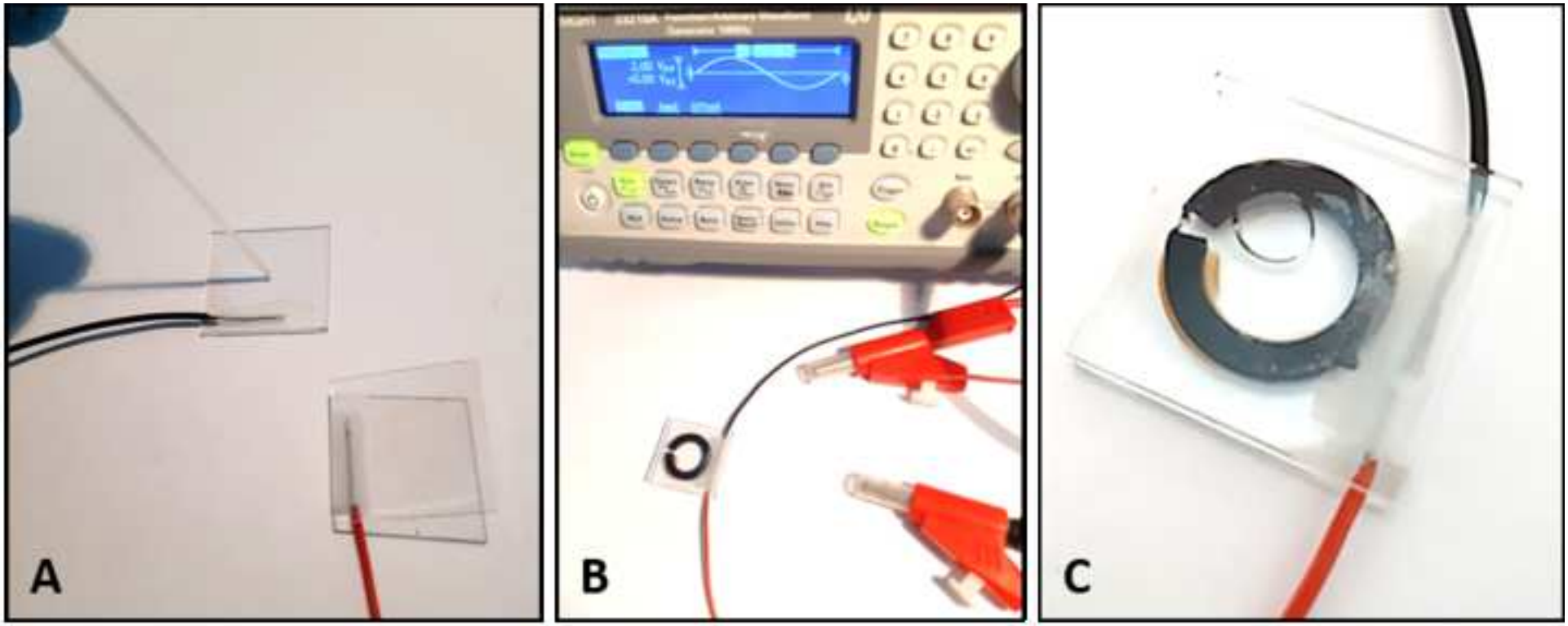

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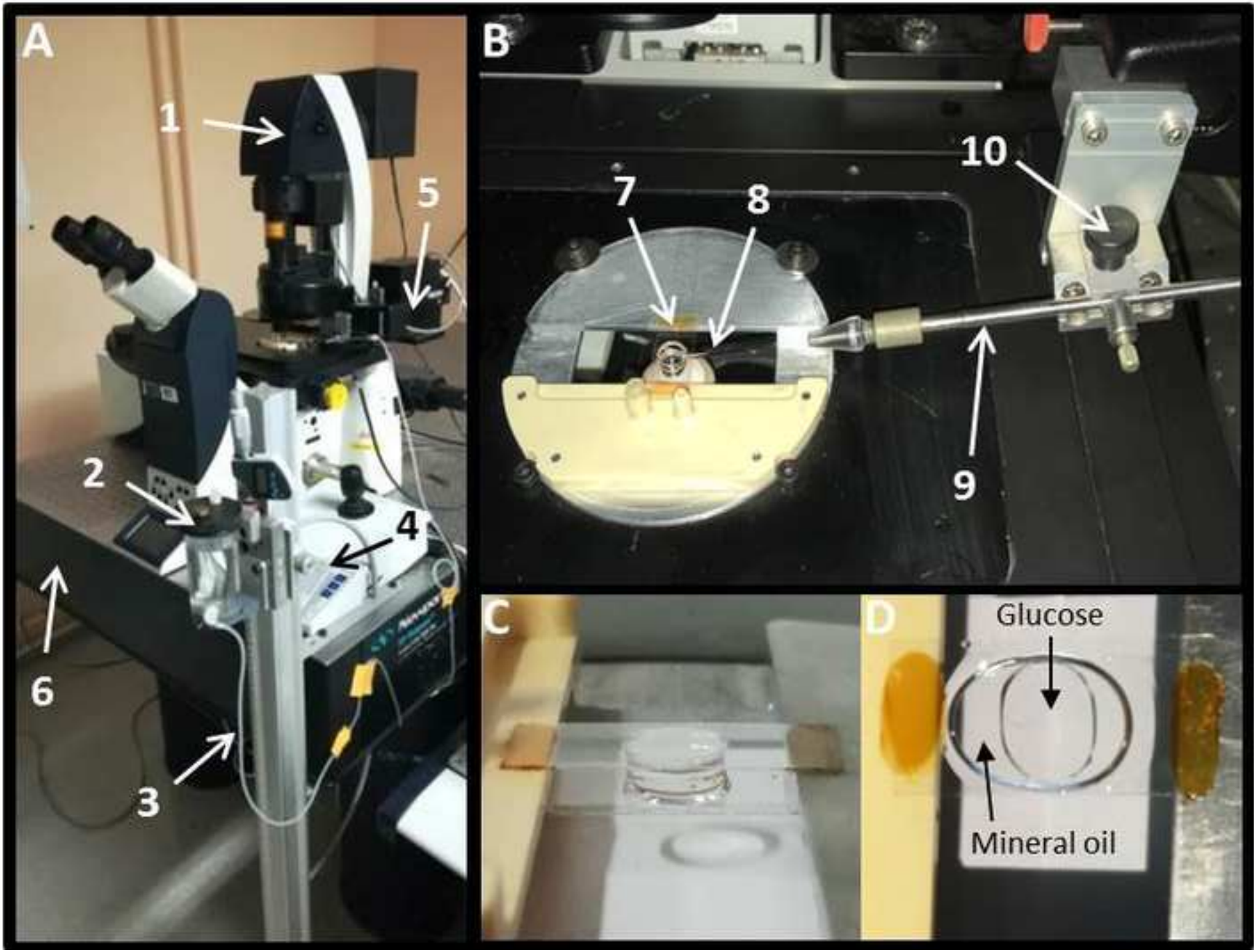

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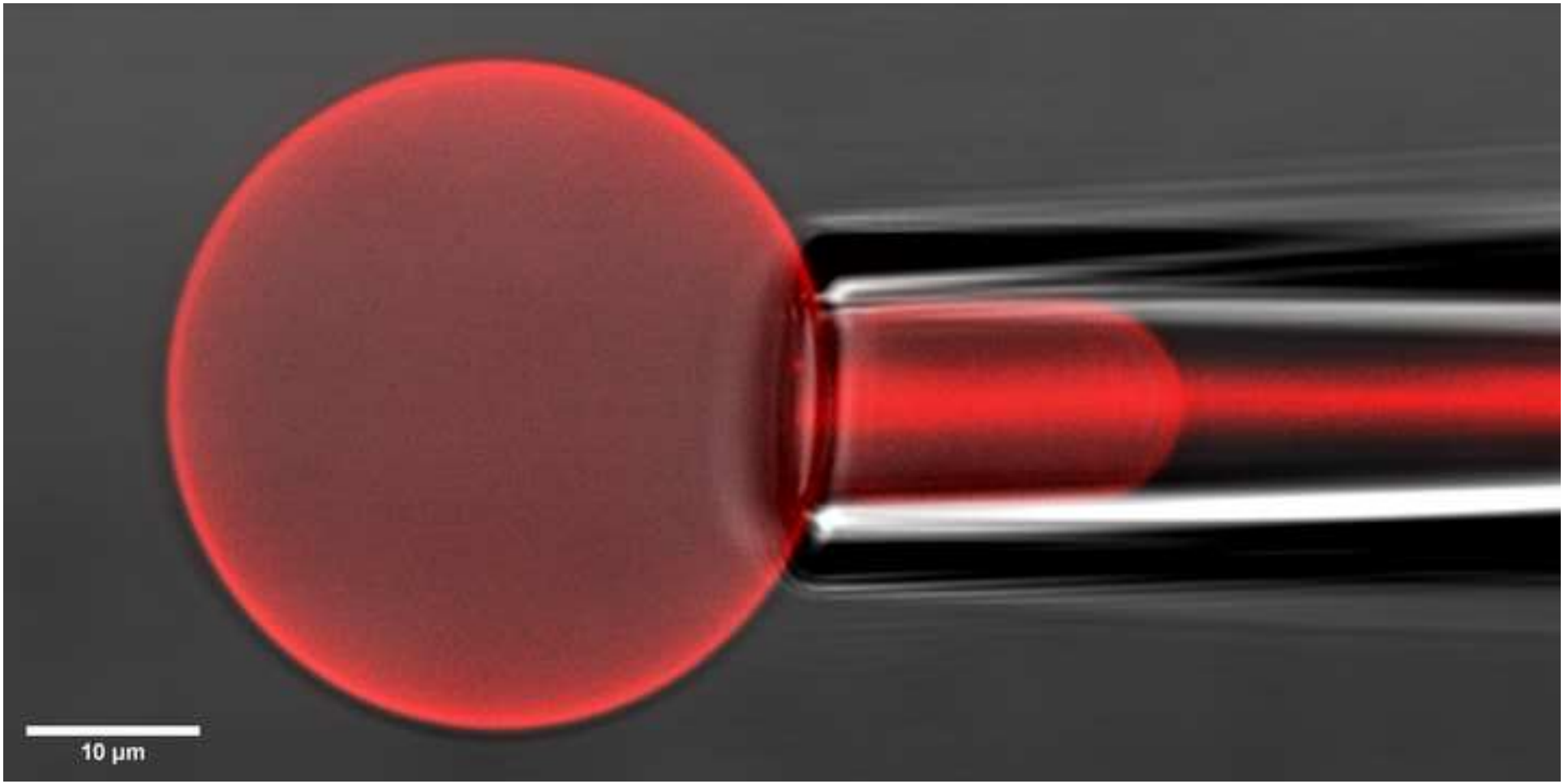

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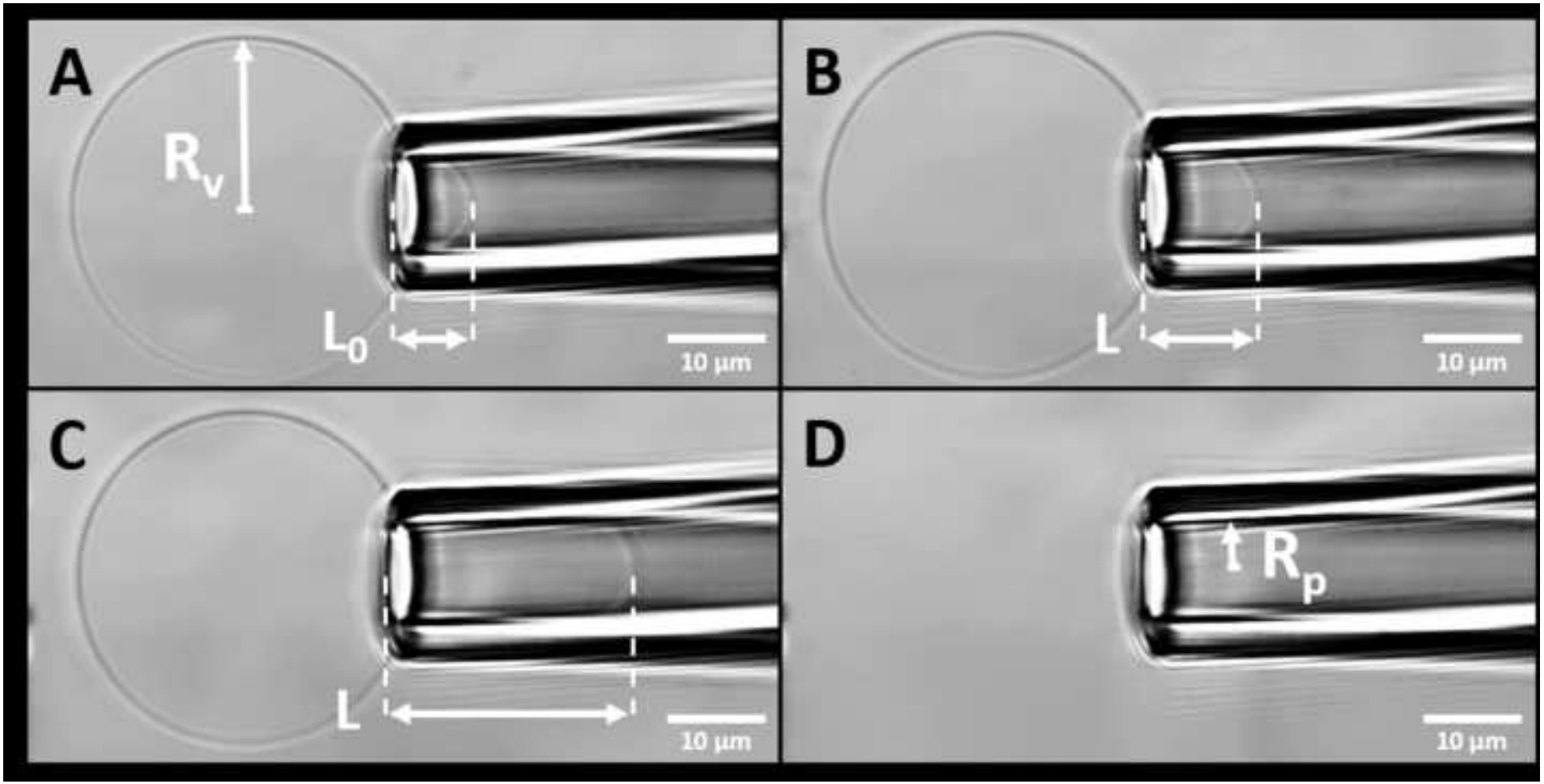

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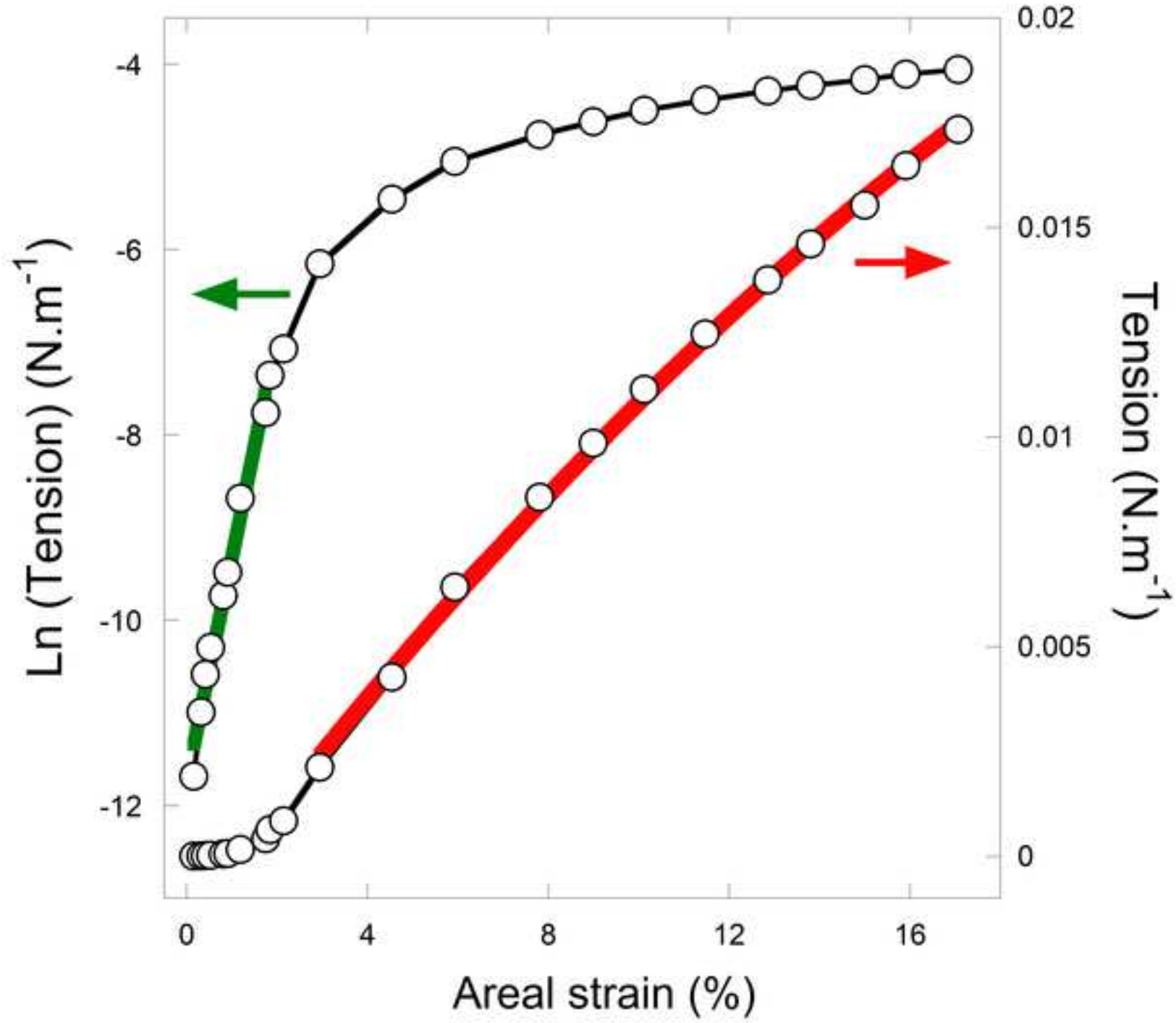

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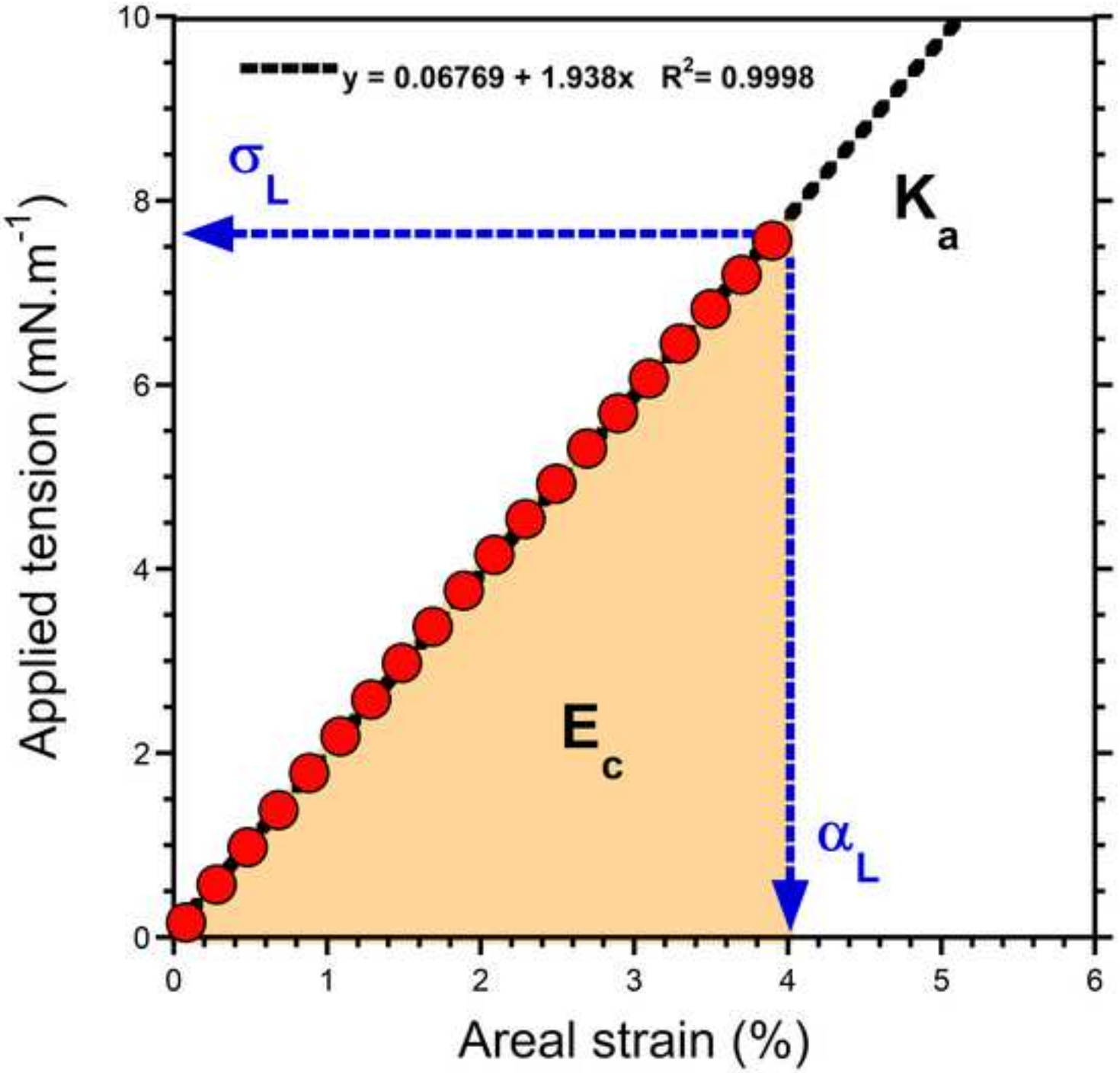

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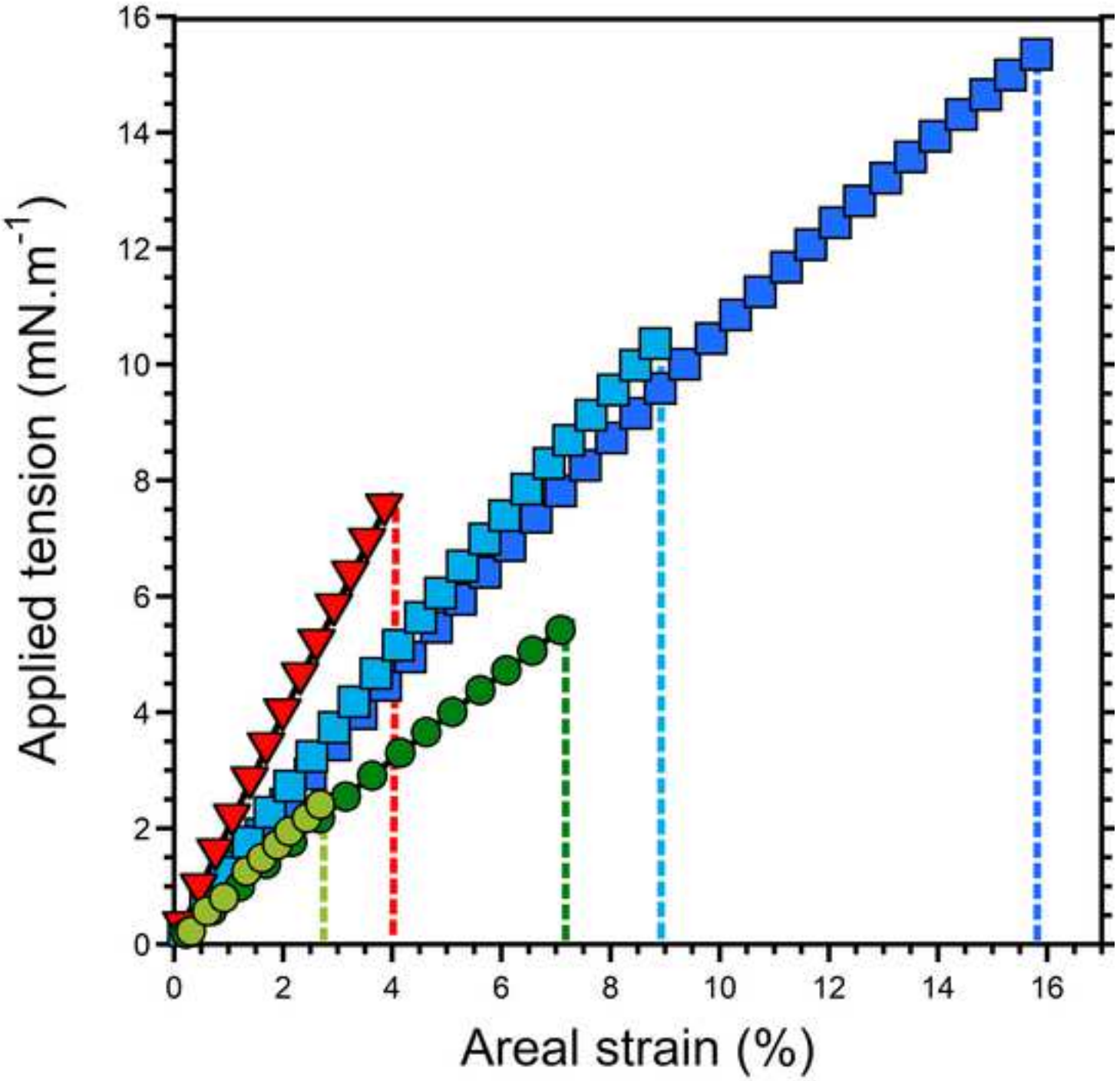

Table

	Ka ($mN.m^{-1}$)	α_L (%)	σ_L ($mN.m^{-1}$)	E_c ($mN.m^{-1}$)	Kb (kT)
POPC	194 ± 15	4 ± 1	8 ± 2	0.17 ± 0.09	21.1±0.4
$PDMS_{27}$-*b*-PEO_{17}	121 ± 8	16 ± 4	15 ± 3	1.37 ± 0.67	10.6 ± 3.5
$PDMS_{27}$-*b*-PEO_{17} + 5 wt.% POPC	132 ± 13	9 ± 4	10 ± 3	0.50 ± 0.38	-
PEO_{12}-*b*-$PDMS_{43}$-*b*-PEO_{12}	84 ± 13	7 ± 1	6 ± 2	0.50 ± 0.38	-
PEO_{12}-*b*-$PDMS_{43}$-*b*-PEO_{12} + 5 wt.% POPC	91 ± 11	3 ± 1	2 ± 1	0.03 ± 0.01	-

Name of Material/ Equipment	Company	Catalog Number
Required equipment and materials for micropipette design **:**		
Borosilicate Glass Capillaries	World Precision Instruments	1B100-4
Filament installed	Sutter Instrument Co.	FB255B
Flaming/Brown Micropipette Puller	Sutter Instrument Co.	Model P-97
Microforge	NARISHGE Co.	MF-900
Materials for coating pipette tips with BSA:		
Bovine Serum Albumin Fraction V (BSA)	Sigma-Aldrich	10735078001
Disposable 1 ml syringe Luer Tip	Codan	62.1612
Disposable 10 ml syringe Luer Tip	Codan	626616
Disposable 5 ml syringe Luer Tip	Codan	62.5607
Disposable acetate cellulose filter	Cluzeau Info Labo	L5003SPA
Flexible Fused Silica Capillary Tubing	Polymicro Technologies.	TSP530660
Glucose	Sigma-Aldrich	G5767
Syringe 500 µL luer Lock GASTIGHT	Hamilton Syringe Company	1750
Test tube rotatory mixer	Labinco	28210109
Micromanipulation Set up		
Aluminum Optical Rail, 1000 mm Length, M4 threads, X48 Series	Newport	
Damped Optical Table	Newport	
Micromanipulator	Eppendorf	Patchman NP 2
Micrometer	Mitutoyo Corporation	350-354-10
Plexiglass water reservoir (100 ml)	Home made	
TCS SP5 inverted confocal microscope (DMI6000) equipp	**Leica**	
X48 Rail Carrier 80 mm Length,with 1/4-20, 8-32 and 4-40 thread	Newport	

Materials for sucrose and amphiphile solution preparation

2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine	Sigma-Aldrich	
Chloroform	VWR	22711.244
L-α-Phosphatidylethanolamine-N-(lissamine rhodamine B sulfonyl)	Sigma-Aldrich	810146C
Sucrose	Sigma-Aldrich	S7903

Electroformation set up

10 µL glass capillary ringcaps	Hirschmann	9600110
Disposable 1 ml syringe Luer Tip	Codan	62.1612
H Grease	Apiezon	Apiezon H Grease
Indium tin oxide coated glass slides	Sigma-Aldrich	703184
Needle	Terumo	AN2138R1
Ohmmeter (Multimeter)	Voltcraft	VC140
Toluene	VWR	28676.297
Voltage generator	Keysight	33210A

Comments/Description

external and internal diameter of 1mm and 0.58 mm respectively.
2.5mm*2.5mm Box Filament

fitted with two objectives (10x and 32x)

Pore size: 0.22µm, diameter: 25mm
Inner Diameter 536µm, Outer Diameter 660µm,

used as support of microscope to prevent external vibrations.
The module unit (motor unit for X, Y and Z movement) is mounted on the inverted microscope by the way of an adapter.
Digimatic LCD Micrometer
Head 25,4 mm Range
0,001 mm

Rhodamine tagged lipid

Silicon-free grease

0.8 x 38 mm

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Author(s): Emmanuel Ibarboure, Martin Fauquignon, Jean-François Le Meins

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