

Bursting of charged multicomponent vesicles subjected to electric pulses

Supplementary Information

Karin A. Riske,^a Roland L. Knorr,^b and Rumiana Dimova^{b,*}

^a Depto. Biofísica, Universidade Federal de São Paulo, R. Botucatu, 862 CEP 04023-062 São Paulo, Brazil;

^b Max Planck Institute of Colloids and Interfaces, Science Park Golm, 14424 Potsdam, Germany

* Address for correspondence: rumiana.dimova@mpikg.mpg.de

Lipid extraction from red blood cells

Fresh human red blood cells (RBC) (courtesy of Nina Geldmacher from the Max Planck Institute for Infection Biology, Berlin) were purified according to the method described in (1). The RBC were washed 4 times in cold isotonic buffer (145 mM NaCl, 5 mM KCl, 5 mM Hepes, pH 7.4 at 4 °C) and centrifuged 10 min at $2000 \times g$ at 4 °C. Then, the cells were lysed by shaking 10 min on ice in hypotonic solution (15 mM KCl, 0.01 mM EDTA, 1 mM EGTA, 5 mM Hepes, pH 6.0) to reduce premature, spontaneous resealing of the ghosts. EDTA, EGTA and Hepes were purchased from Sigma-Aldrich, Germany. The ghosts were washed once in hypotonic solution without EGTA and twice in hypotonic solution without EGTA but with 2 mM Mg^{2+} and centrifuged for 10 min at 4 °C and $12000 \times g$. To remove peripherally associated proteins (and remaining hemoglobin, which is known to contaminate lipid extracts (2)) from the membrane of the open RBC ghosts, they have been shaken for 30 min on ice in 10 mM NaOH and centrifuged. The lipids were extracted from the obtained pellet using the method of Bligh and Dyer (3). Briefly, the pellet was diluted with bidistilled water to a volume of 240 μ l, followed by stepwise addition and vortexing of the following solutions: 300 μ l chloroform, 600 μ l methanol, 300 μ l chloroform, 300 μ l bidistilled water. After 5 min centrifugation of the obtained solution at $2000 \times g$, the chloroform-rich phase was separated, stored at -20°C and used for vesicle preparation.

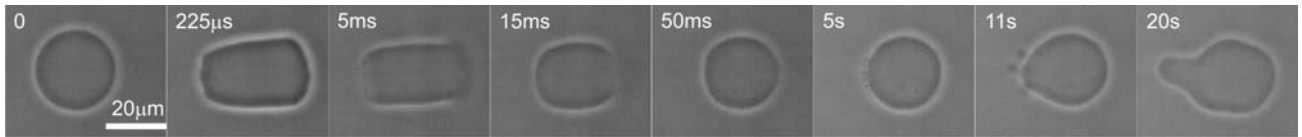
The lipid extract obtained according to the protocol of Bligh and Dyer (3) is known to contain about 10 % phosphatidylserines, 14 % phosphatidylcholines, 14 % phosphatidylethanolamines, 11 % sphingomyelins, and 50 % cholesterol (4).

Vesicle preparation and observation

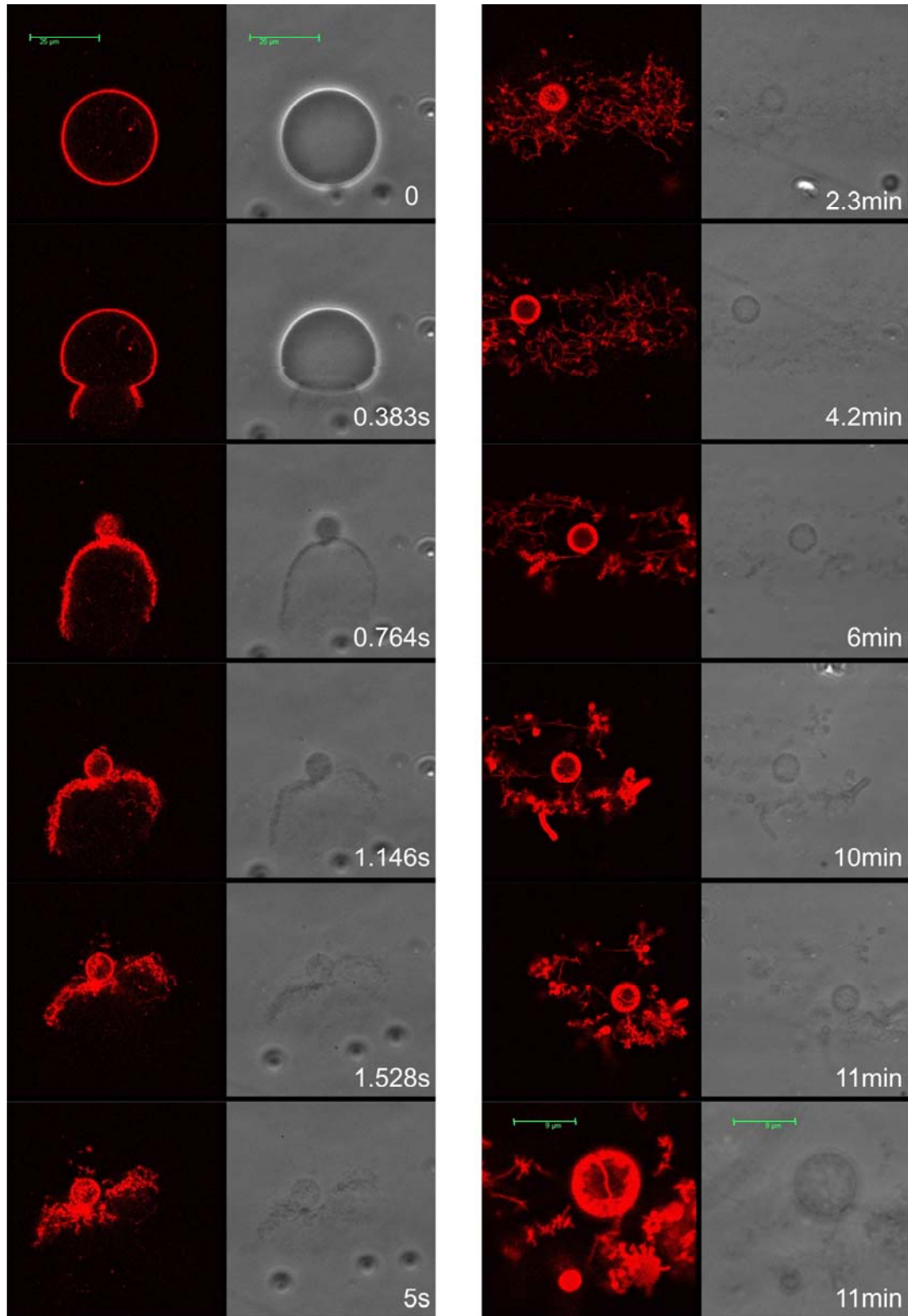
Giant unilamellar vesicles were prepared from palmitoylcholine (PC), palmitoylphosphatidylcholine (PG) and palmitoylphosphatidylethanolamine (PE) (Avanti Polar Lipids, Alabaster, AL) or lipid extract (LE) from the RBC plasma membrane using the electroformation method (5). PG and PC were mixed in molar ratios 1:9, 2:8 and 1:1. Only the 1:1 molar ratio was explored for mixtures of PE and PC. For the samples used for confocal microscopy, up to 0.2 mol % of dipalmitoylphosphatidylethanolamine-Rhodamine (Rh-DPPE) or 0.5 % of 1-Oleoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-sn-Glycerol-3-[Phospho-rac-(1-glycerol)] (Ammonium Salt) (NBD-PG) from Avanti Polar Lipids were incorporated in the membrane. No change in the vesicle behavior was observed depending on the type of dye and compared to the dye-free vesicles.

The procedure for vesicle electroformation is described in detail in (6). Briefly, 15 μ l of a 2 mg/ml lipid solution in chloroform were spread on the surfaces of two conductive glasses (coated with Indium Tin Oxide). To prepare vesicles to be observed with confocal microscopy 0.2 mol% fluorescent dye was included in the lipid chloroform solution. The glasses with the lipid film were kept under vacuum for about 2 h to remove all traces of the organic solvent. The two glasses separated by a 2 mm thick Teflon frame were assembled to form a chamber sealed with silicon grease. The glass plates were connected to an AC field function generator. The chamber was filled with 0.2 M sucrose solution (referred to as non-buffered solution in the article). In some samples, we added 1 mM Hepes and 0.1 mM EDTA, pH 7.4 (buffered solution). In others, we added 0.5 mM NaCl (salt solution), which provides the same ionic strength as the buffered solutions. An alternating current of 1 V at 10 Hz frequency was applied for 1 h. Vesicles with an average diameter of 50 μ m and a large polydispersity were observed to form. The vesicle suspension was diluted 20 times into 0.2 M glucose solution (again in certain cases containing the above-mentioned buffer and salt solutions), thus creating sugar asymmetry across the vesicle membrane. The osmolarities of the inner and the outer solutions were measured with a cryoscopic osmometer Osmomat 030 (Gonotec, Germany) and carefully matched to avoid osmotic stress.

After dilution, the vesicles were transferred to a commercially available electrofusion chamber (Eppendorf, Hamburg, Germany). The latter consist of two parallel cylindrical electrodes of about 92 mm radius with 500 μ m gap distance. Due to the density difference between the sucrose and glucose solutions, the vesicles settled at the bottom of the observation chamber where they were easy to locate. The refractive index difference produced good optical contrast for phase contrast observation: the vesicles appear as dark objects on a light gray background. The leakage through micrometer pores could be visualized by the dark sucrose leaving the vesicles. Electric pulses were generated by a Multiporator (Eppendorf). Videos were recorded with fast imaging camera HG-100 K (Redlake, San Diego, CA) or confocal SP5 system (DMI 6000, Leica Microsystems Heidelberg GmbH, Germany) equipped with a $40 \times$ HCX Plan APO objective (NA 0.75). Laser sources at 561 nm and 488 nm were used to excite Rh-DPPE and NBD-PG, respectively. The acquisition speed using the fast camera was 20 000 frames per second (fps), while with the confocal microscope it was approximately 3 fps. Because of the slow scanning speed for the confocal microscopy observations, snapshots immediately after the pulse may be distorted in the field direction due to the slow scanning in lines perpendicular to it. The following figure provides the snapshot sequence in Fig. 1 in the main text, here supplemented with an additional image demonstrating that the formed pore is closed within the first 50 ms:



Below we include more images with better resolution of the vesicle from Fig. 2 in the article. The membrane was labeled with Rh-DPPE. The scale-bar on all but the last images corresponds to 25 μm . The last image is refocused and magnified with scale-bar corresponding to 9 μm .



REFERENCES

1. A. D. Dupuy and D. M. Engelman, *Proc. Natl. Acad. Sci. U.S.A.*, 2008, 105, 2848-2852.
2. H. G. Rose and M. Oklander, *J. Lipid Res.*, 1965, 6, 428.
3. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, 1959, 37, 911-917.
4. T. Nakano, Y. Wada and S. Matsumura, *Clin. Hemorheol. Micro.*, 2001, 24, 85-92.
5. M. I. Angelova and D. S. Dimitrov, *Faraday Discuss. Chem. Soc.*, 1986, 81, 303-311.
6. K. A. Riske and R. Dimova, *Biophys. J.*, 2005, 88, 1143-1155.