Electroporation and membrane fusion of charged lipid vesicles

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Several methods of intracellular delivery of materials rely on a first step which consists of crossing the biological membrane. Among them, electroporation and fusogenic liposomes have proven to be effective in delivering materials to which cells are normally impermeable. In electroporation, one or more intense electrical pulses are applied and the plasma membrane becomes transiently permeable, allowing substances to enter the cell. Fusion between liposomes and cells results in the delivery of both encapsulated and incorporated substances to the cytosol and plasma membrane, respectively. Here we use giant unilamellar vesicles (GUVs) as biomimetic models of cells to simulate real systems in a controlled way. A series of advanced microscopic techniques are used to study electroporation and membrane fusion in detail. Macropores induced by DC pulses in neutral GUVs are transient, with a life span of hundred milliseconds. In contrast, pores formed in negative GUVs can open indefinitely, leading to total collapse of the vesicle, in a process known as bursting. Here, we quantify the fraction of destabilized GUVs and the pore edge tension as a function of the fraction of PG in the membrane, the degree of lipid unsaturation and certain additives of the medium. The fusion of cationic liposomes with PC:PG GUVs is studied in a new LUV-GUV fusogenic system, in which the fusion is observed in real time and its effects directly observed and quantified in the microscope. Förster Resonance Energy Transfer (FRET) assays are used to measure the amount of lipids transferred from LUVs to GUVs, and show that hemifusion-fusion transition occurs at 10-20 mol% PG in the acceptor GUV system. In all cases, fusion efficiency is driven by neutralization of charges.