

**Electroformation of Giant Unilamellar Vesicles from Damp Films for Lipid Mixtures Containing High Cholesterol Content, Charged Lipids, and Buffers as Internal Solutions**

Marija Raguz<sup>1</sup>, Ivan Mardešić<sup>2</sup>, Krešimir Kuliš<sup>3</sup>

<sup>1</sup> University of Split School of Medicine/Croatian Biophysical Society, Split, Croatia

<sup>2</sup> University of Split School of Medicine, Split, Croatia

<sup>3</sup> University Hospital of Split, Split, Croatia

Giant unilamellar vesicles (GUVs) are frequently used as models in studies of membrane properties. They are usually produced using the electroformation method. A negative effect on GUV electroformation have the presence of high cholesterol (Chol) content, charged lipids, and buffers. The traditional electroformation protocol involves drying of the lipid film where anhydrous Chol crystals form. If Chol concentration is high it will cause the issue of artifactual Chol demixing. These crystals are not involved in the formation of the lipid bilayer, resulting in a lower Chol concentration in the bilayer compared to the original lipid mixture. Motivated primarily by this issue, we have modified the electroformation protocol by incorporating the techniques of rapid solvent exchange, ultrasonication, plasma cleaning, and spin-coating for reproducible production of GUVs from damp lipid films. A high yield of GUVs was obtained for Chol/1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) samples with mixing ratios ranging from 0 to 2.5. We also succeeded in preparing GUVs from mixtures containing up to 60 mol% of the charged lipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) and in buffers. Aside from decreasing Chol demixing, we have shown that the method can also be used to produce GUVs from lipid mixtures with charged lipids and in buffers used as internal solutions.