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## Hybrid Phospholipid Bilayer surface properties investigation and modification using Scanning Electrochemical Microscopy

Margarita Poderytė\*, Inga Gabriūnaitė, Aušra Valiūnienė

Faculty of Chemistry and Geosciences, Vilnius University, Naugarduko str. 24, Vilnius, Lithuania \*margarita.poderyte@chf.stud.vu.lt

An important component of biological cells is its membrane, which separates the cell from the environment and regulates the exchange of substances inside the cell and vice versa.<sup>1</sup> Since cell membranes are complex systems, models are developed to replicate their properties. One of the widely investigated and modified membrane properties is selective permeability, which can be increased by creating adjustable size pores.<sup>1,2</sup> Scanning Electrochemical Microscopy (SECM) is a fast-growing technology in biological research in which signal is Faradaic current of the tip - ultramicroeletrode (UME). Whereas the size of the UME used vary from few nm to 25  $\mu$ m, measurements for very small samples with high resolution data are possible.<sup>3</sup> Using SECM different operation it becomes possible with only one instrument investigate the surface properties of the membrane, modify it, and detect changes.<sup>3,4</sup>

In this work a glass plate coated with a layer of tin oxide doped with fluorine atoms (FTO) was used as a substrate for self-assembled monolayer (SAM) and phospholipid bilayer membrane (BLM) formation. A mixture of octadecyl trichloro silane (OTS) and vinyltrimethoxy silane (VTS) was used to form SAM. The hybrid phospholipid bilayer membrane was prepared using the vesicle fusion method from 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and cholesterol in molar % ratio 6:4 in phosphate buffer solution (PBS) containing 0.1M NaCl, 0.01M NaH<sub>2</sub>PO<sub>4</sub>, pH 4.6.<sup>5</sup>

Electrochemical measurements were carried out using three electrode system where platinum UME ( $\emptyset$  25 µm) was used as working electrode, platinum wire as counter and Ag/AgCl/KClsat as reference. Surface investigation measurements were performed with scanning electrochemical microscope using a feedback mode. Carrying out many targeted approach curve experiments on surface, SECM is used to locally detect membrane defects and the effect of toxin. Characteristic feedback curves of clean, unmodified FTO glass surface was compared to curves of (i) SAM-modified FTO; (ii) a phospholipid membrane-coated FTO prior to interaction with the toxin phospholipase and (iii) a phospholipid membrane-coated FTO after phospholipase interaction with the phospholipid membrane.

BLM electroporation was performed by applying a 1 V voltage to the working electrode for 1.5 minute at a distance of  $5\mu m$  from the surface. Approach curves before and after electroporation were compared to detect changes of surface. Since the surface of membrane is more or less heterogeneous, it has been observed that at different points, the electroporation phenomenon with discussed conditions ensues differently.

Whereas different size of tip may be used in scanning electrochemical microscopy there is a great chance that adjustable size of pores may be obtained. Moreover, measurements can be held in a bio-compatible environment. This leads to a promising expectation that SECM has a great potential in investigation of both biological cell membranes and their models.

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