

Development of solid supports for electrochemical study of biomimetic membrane systems - DTU Orbit (09/11/2017)

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Biomimetic membranes are model membrane systems used as an experimental tool to study fundamental cellular membrane physics and functionality of reconstituted membrane proteins. By exploiting the properties of biomimetic membranes resembling the functions of biological membranes, it is possible to construct biosensors for high-throughput screening of potential drug candidates. Among a variety of membrane model systems used for biomimetic approach, lipid bilayers in the form of black lipid membranes (BLMs) and lipo-polymerosomes (vesicle structures composed of lipids and polymers), both with reconstituted membrane spanning proteins, are attractive tools. However, BLMs suffer from intrinsic fragility, therefore, requiring techniques to increase their robustness and stability. This PhD thesis presents strategies to construct solid supports for electrochemical studies of two biomimetic membrane systems, BLMs and protein-loaded lipopolymerosomes.

The solid support for BLMs was constructed as a reusable device comprising an ethylene tetrafluoroethylene (ETFE) aperture array supported by an in situ polymerized hydrogel covalently attached to both the ETFE and a gold electrode microchip. The hydrogel facilitated BLM formation without the need of manual painting and increased membrane stability in comparison with freestanding membranes. The functionality of the hydrogel supported BLMs (hsBLMs) were demonstrated by electrochemical impedance spectroscopic (EIS) characterization of incorporated ion transporter valinomycin. The presented work also includes a comprehensive EIS analysis and cryological scanning electron microscopic (cryo-SEM) imaging of hydrogels formulated in various molar ratios (1:100; 1:200; 1:400) of the cross-linker poly(ethylene glycol)dimethacrylate (PEGDMA) and 2-hydroxyethylene methacrylate (HEMA) monomers.

Lipo-polymerosomes have proved to be suitable for reconstitution of a model G-protein coupled receptor (GPCR) - bacteriorhodopsin (bRh). The bRh-loaded lipo-polymerosomes were interfaced to gold electrodes using two different strategies, layer-by-layer deposited polyelectrolyte cushion directly on a gold electrode microchip and on a polyethersulfone (PES) support grafted by in situ polymerized hydrogel. Both strategies proved to be suitable for immobilization of functional bRh loaded lipo-polymerosomes. Amperometric monitoring showed that the PES membrane support facilitated recording of a steady-state photocurrent while only a transient photocurrent peak was recorded on the polyelectrolyte cushion without a PES membrane.

This PhD thesis also comprises the design and fabrication process of a modular microfluidic system with automated fluid delivery (micropumps and valves), providing a possibility for future applications of biomimetic membranes in the form of hsBLMs and polymerosomes.

This thesis presents both strategies for formulation robust biomimetic membrane systems and devices, which could be developed further to construct biosensor technology for high-throughput screening of drug candidates.

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