ABSTRACT

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Nerve cells release small packets of neurotransmitters from membrane-enclosed vesicles upon stimulation. This release mechanism is of central importance in neuronal communication and thus in brain function. The focus of this thesis is to develop new techniques and model systems to improve our understanding of the mechanisms of exocytosis. The thesis presents three novel techniques. The first of these is a quartz crystal microbalance with dissipation (QCM-D) technique to measure exocytotic and endocytotic events from an ensemble of secretory cells. A second part of the thesis involves development of liposome-nanotube networks and techniques to create these structures. These systems are demonstrated to be suitable for the study of dynamic events such as shape transformations of biomembrane systems and controlled material transport. In the third part of this thesis, liposome-nanotube techniques have been used to create an "artificial cell" that can mimic the later-stage of exocytosis. Release of catecholamine was detected using amperometry.

In **Paper I,** the QCM-D sensor is presented as a means to measure the dynamical changes in mass during exocytosis and subsequent endocytosis in small populations of neuronal-like cells following stimulation. Changes in the energy dissipated and resonance frequency due to translation of the cell material when the crystal frequency is turned off, were measured for stimulated release at PC12 and NG 108-15 cells. This was shown to relate to the viscoelastic changes in the cell layer during exo- and endocytosis.

Techniques for construction of liposome-nanotube networks are presented and transport in these systems is demonstrated in **Paper II**. A micropipette-assisted technique for constructing liposome-nanotube networks from unilamellar liposomes is introduced in **Paper III**. The network was differentiated by microinjecting different materials into individual liposome compartments. In **Paper IV**, the construction of geometrically complex liposome-nanotube networks is presented. Existing micropipette-assisted techniques were combined with a microelectrode-based electrofusion technique in this work. The forces exerted on the release of satellite-vesicles have been studied. In **Paper V**, tension-driven transport in vesicle-nanotube networks is discussed.

In **Paper VI,** an artificial cell, which consists of a transmitter-filled vesicle inside a liposome with a connecting lipid nanotube, is presented. This model system imitates the later stages of exocytosis and was used to show that membrane mechanics can drive dilation of the fusion pore without protein intervention. Amperometry was used to measure the release of catechol from vesicles and was also used to measure leakage of transmitter analogue through the fusion pore. This leads to **Paper VII,** where data is presented with the liposome model and electrochemical detection to form an "artificial synapse". A carbon fiber electrode was placed in close proximity to an artificial cell and release during exocytosis was measured as in Paper VI. The ultra-thin space between the electrode and the membrane was used to model amperometic measurements and release in synapses.

Key words: **Exocytosis, endocytosis, quartz crystal microbalance, QCM-D, dissipation, liposome, lipid bilayer nanotube, nanofluidic, amperometry, artificial cell, artificial synapse**
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