613c Arraying of Intact Liposomes on Chemically Functionalized Microwell Surfaces with Potential Application as Biosensors

Nikhil D. Kalyankar, Manoj K. Sharma, M. L Gilchrist, David Calhoun, Charles Maldarelli, and Alexander Couzis

We are developing protocols to array individual, intact small unilamellar vesicles (liposomes) onto surfaces with potential application as biosensor probes. One potential problem in arraying of liposomes on surfaces is their tendency to unravel upon adsorption. We tackle this problem by situating liposomes inside microwells chemically functionalized to attract liposomes. The size of liposomes is matched with the size of the wells on micro-patterned surface so that only one liposome attaches into each well. The background is functionalized to resist liposome adsorption and unraveling. In the ongoing research, the surfaces used are microwell arrays with 1.2 μm diameter fabricated by Photolithography on a silicon wafer coated with silicon oxide layer. A flat block of Polydimethylsiloxane (PDMS) impregnated with Polyethylene glycol (PEG) terminated silane is used to put down a PEG-terminal background phase using Contact Printing. PEG terminated monolayers are resistant to protein/liposome adsorption. The ‘bare' holes are then backfilled using amine terminal 3-Aminopropyltrimethoxysilane (APS). These amine microwells are biotinylated using NHS-PEO4-Biotin (NPB). Next Neutravidin is attached to the Biotin islands to form patterned Neutravidin arrays capable of binding more Biotin. Low Tg lipid formulations containing 5% biotinylated lipids are used to prepare liposomes of about 1 μm diameter using an Extrusion technique. The patterned Neutravidin microwell array is then exposed to the liposome solution, which results in attachment of intact liposomes into holes by ‘Biotin-Neutravidin' interaction. The intactness of liposomes after attachment is verified by co localization of fluorescence from cargo incorporated inside the liposomes with the fluorescence from Neutravidin grid using Confocal Microscopy. Other steps involved in the protocol are confirmed using Atomic Force Microscopy, Fluorescence Microscopy, Particle Size Analysis, and Confocal Microscopy.