EX-VIVO BIOMIMETIC INTERFACES FOR SCREENING ENGINEERED NANOMATERIALS

Engineered nanomaterials (ENM) have attractive functional properties and are increasingly being used in commercial products. However, the potential health risks induced by ENM are poorly understood and difficult to evaluate. Since the first step in ENM toxicology pathway is to interface with cell membranes to trigger biological effects, improved methods are needed to measure ENM-biomembrane interactions. Therefore, the overall objective of my doctoral dissertation is to develop robust, high throughput nano-structured biosensors, to elucidate mechanisms of surface interaction between ENM and biomembrane, to evaluate ENM kinetics and performance, to predict ENM induced health risk, and to provide guideline for bio-safety design of nano-products with desired functions.

The first generation of higher throughput interface was developed by accelerating the interface-assembly process and enhancing stability of a planar bilayer lipid membrane (pBLM). The diameter of a classical planar BLM is ~500 µm. The novel planar BLM developed for this project was fabricated on nanopores (~700 nm) drilled through silicon nitride thin film using focused ion beam
lithography. The resulting nano-biosensor enabled automatic BLM formation after integration into a microfluidic device with enhanced mechanical stability. Electrophysiology and electrochemical impedance spectroscopy was used to validate functionality of the nanopore pBLM. Then, transient current spikes induced by silica nanoparticles and integral conductance induced by carboxylate multi-wall carbon nanotubes, across giga-ohm nanopore pBLM, were measured. The chronoamperometric traces have single-pore sensitivity and temporal resolution on the order of millisecond. However, the first generation BLM requires manual formulation and expensive screening equipment, limiting its potential of commercialization.

As a result, the second generation of high throughput biomimetic interface, tethered BLM on gold electrode was developed. Immobilization of BLM on surfaces offers advantages over pBLM, including enhanced stability and ion reservoir. Another advantage associated with tethered BLM is that it can be integrated onto complementary metal–oxide–semiconductor based microsystem with on-chip electrochemical detection. Using molecular self-assembly, the resulting microelectrode array based tethered BLM forms a continuous use and label-free detection platform that is suitable for microsystem implementation. The model interface, tethered BLM, verifies the viability of biocompatible fabrication technologies and is a milestone toward integrating electrochemical biosensor arrays and microelectronic instrumentation into high throughput, manual free and cost effective drug screening applications.

Here, pore forming activities of functionalized silica nanoparticles, functionalized polystyrene nanoparticles (PNP) and functionalized polypropargyl glycolide nanoparticles (PGL) were characterized using high insulating tethered BLM. Electrical resistance trajectory was analyzed using empirical exponential model, providing dynamic information of ion leakage induced by various nanoparticles. Coupled with statistical hierarchical clustering post-analysis, the tethered BLM method could distinguish between nanoparticles based on size, charge and/or surface functional
groups by monitoring dynamic electrical resistance and overall resistance loss. However, the
exponential model failed to reproduce the resistance trend for –OH terminated PGL.

As a result, a mechanistic kinetic model was developed to predict interaction kinetics of
ENM with model BLM and to correlate effects of different component, and to assess biosensor
performance. A 9 parameter model was designed using MATLAB and R, and then simplified into a 3
parameter model. The model developed helps elucidate molecular processes responsible for time-
evolved electrochemical property/signal changes in tethered BLM following ENM exposure.
Assuming second order reaction kinetics, the model uses two kinetic constants to describe the rates
of ENM binding to, and lipid removal from, the interface. The rates were dependent on number of
free nanoparticles, available site on the interface and mobile lipids fraction. The model was fit to
membrane resistance, $R_m$ vs. time data for interaction of a tethered BLM with two types of ENM:
PGL and functionalized polystyrene nanoparticles. The model was able to predict diverse trends in
the $R_m$ vs. time data, including a continuous decrease (PNP) in $R_m$ and an initial increase (PGL) in
$R_m$ followed by a decrease. The model also predicted that the biosensor is so sensitive that the
interaction involving only $10^{-8}$% of bulk nanoparticles could generate measurable changes in the
biosensor output. Then, kinetic constants for ENM extracted from time-evolved trends were analyzed
using hierarchical clustering. The resulting dendrograms showed that ENM with different properties
(composition, size, surface charge) could be statistically distinguished using this approach.

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