Investigating mechanisms at the cell membrane-nanoneedle interface for directing intracellular entry of biological cargo

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Abstract

Introduction
Obtaining intracellular access has been a challenge for majority of *in-vitro* and *in-vivo* cell manipulations involving biomolecule delivery and sensing. Our group has recently developed biodegradable porous silicon nanoneedles (nN) as a platform for intracellular sensing and improved efficiency of delivery by providing access to the cytosol[1-3].
Interfacing cells with high aspect ratio nN affects membrane architecture, dynamics and conformation, leading to improved association between the membrane-nN payload[2]. However, it is still unclear how biomolecules are transferred across the membrane, whether they enhance cell uptake processes or make direct contact with the cytosol.

Methods
In this investigation, we studied the interactions of hMSCs with nN arrays. nN arrays created in our lab were interfaced with hMSCs at various time points. We analysed cell membrane architecture on nN arrays by scanning electron microscopy (SEM) and live scanning ion conductance microscopy (SICM). Cell uptake mechanisms were analysed by immunocytochemistry and western blotting. Integrity of the cell membrane was studied using focused ion beam scanning electron microscopy (FIB-SEM).

Results
In comparison to flat silicon wafers (FSW), hMSCs cultured on nN showed remarkable changes in membrane conformation as membrane appeared to be significantly more ruffled with a large number of invadopodia. In addition, analysis of major cell uptake pathways such caveolin mediated endocytosis suggests that caveolae could play a key role in transporting biomolecules across the membrane as caveolae pits are recruited to sites of nN interfacing. FIB-SEM images of cross-sections of hMSCs on nN showed that membrane integrity was preserved, along with tighter association of the membrane and nN substrate compared to FSW.

Discussion and Conclusions
Interfacing hMSCs with nN affects cell membrane dynamics by inducing membrane ruffling, endocytotic vesicle recruitment while preserving membrane integrity. Understanding the mechanisms involved in biomolecule transport across the membrane barrier during nN interfacing offers insight into how novel materials for can be optimised for successful intracellular manipulations.

References:
[1]C. Chiappini et al (2015) *Nat Materials*
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