Cell based biosensor approach to characterize interactions between nanoparticles and cell surface receptors

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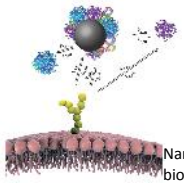
Background

- When nanomaterial come into contact with complex biological fluids, they rapidly get covered by a selected groups of biomolecules and this layer act as the interface between nanomaterial and the environment[1].
- In particular protein molecules get adsorbed onto the surface of nanomaterial and form a coating around the nanomaterial, which refers as 'protein corona' .



Nanoparticles-corona complex in a biological environment [1]

The protein corona signifies the biological identity of the nanomaterial thus effect their applications

Despite the rapid increase of the understanding of the bio-nano-interface *in vitro*, its behaviour in biologically relevant environment remains obscure

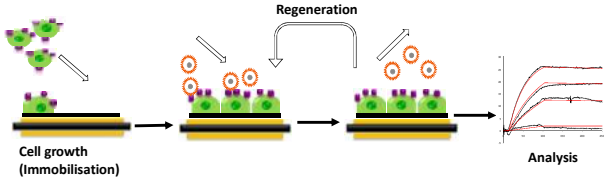
- The work presented is aimed developing a QCM based platform for the characterisation of interactions between bio-nano interface and its cell binding partners using Attana label free cell based biosensor system[2]
- This new biosensor system is based on the quartz crystal microbalance technology (QCM) and utilised adherent cells directly grown on the surface of a sensor in contrast to the conventional biosensor system where the bio receptors are often the purified biomolecules

Methods

Assay set up

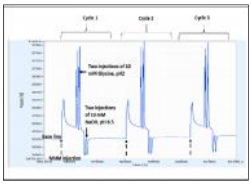
- Adenocarcinoma human alveolar basal epithelial cells (A549) and adenocarcinoma human colon cell line (Caco-2) were selected as model cell lines.
- A549 cells were selected to model the cell exposure to NPs after inhalation and Caco-2 cells were selected as cell exposure via ingestion.
- TiO_2 and SiO_2 nano-particles (50 nm) were used for the assay development

Nanoparticles were sonicated and dispersed in FBS and manually injected over the cell surface



Assay workflow

- Experiments were performed using Attana cell™ 200 biosensor (Attana AB) at a flow rate of 20 ul /min at 22 °C.
- Binding of nanoparticles was studied by sequential injections (105 seconds) of the nanoparticle solutions followed by dissociation up to 200 seconds and the surface was regenerated using 10mM Glycine, pH2 followed by 10mM NaOH at pH 8.5
- The frequency changes during binding experiments were recorded using Attestar software (Attana AB) and analysed using Evaluation software (Attana AB).



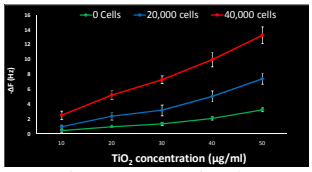
Three sequential injections of TiO_2 nano-particles and regeneration

References

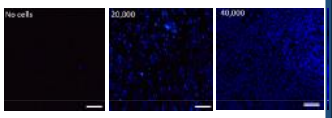
1. Marco P. Monopoli, M.P., Åberg, C., Anna Salvati, A., Dawson, K.A. Nature Nanotechnology. 2012; 7; 779-786
2. Peiris, D., Markiv, A., Curley, G. P., Dwek, M. V.. Biosensors and Bioelectronics. 2012; 35:160– 166
3. Diluka Peiris, Daniel Wallinder, Teodor Aastrup. Eurolab. 2015 (1) 63:6

Results

Effect of seeding density on NPs interactions



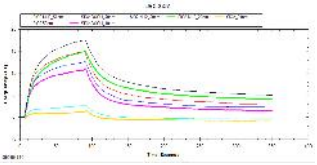
Maximum frequency response of TiO_2 (PROM-TiO_2 un-coated) as a function of seeding density. (Error bars SD for triplicate injections on three different sensor chips with corresponding seeding density)



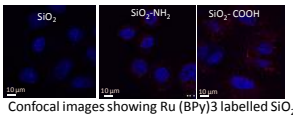
To-Pro3 (1mM) stained A549 cells on QCM sensor surfaces prepared using increasing seeding density. Scale bars 200 µm.

- The maximum frequency response increased with increasing seeding density for all TiO_2 concentrations

Effect of surface modifications on NPs binding

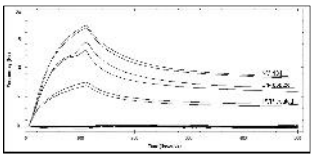


Sensograms for SiO_2 (JRC) binding to A549 cell surface. Amine or carboxyl modified SiO_2 particles showed binding to A549 cell surfaces while unmodified SiO_2 showed no binding at the same conditions.



Confocal images showing Ru (BPy)3 labelled SiO_2 binding to A549 cells.

- Biosensor data is in agreement with the immunofluorescence data, where unmodified Amine or carboxyl modified SiO_2 particles showed binding to A549 cell surfaces



Sensograms for TiO_2 binding to A549 cell surface. Un-coated, PVP coated and rutile hydrophilic (NM104) TiO_2 particles showed binding to cell surface while TiO_2 (NIST), Pluronic F127 coated, Dispex AA4040 coated showed no binding

Particle	kd1	kd2
TiO_2 -uncoated	6.3 E-3	4.98 E-4
TiO_2- (PVP coated)	5.7 E-3	5.31 E-4
TiO_2(rutile, hydrophilic)	5.1 E-3	5.03 E-4

Off rates of TiO_2 particle dissociation

- There were no significant difference between dissociation rate constants of three bound particles. This might imply involvement of same protein from protein corona in the cell surface binding. However this need further investigation using proteomics approach.

Characterization of protein corona

SDS-PAGE analysis of nanoparticles incubated in plasma and FBS

MS analysis of corona proteins – NPs were incubated in FBS

Conclusions & Future work

- The work presented here has successfully demonstrated that Attana QCM cell based system can be utilised to study nanoparticle-protein-cell interactions under *in vivo* biological conditions.
- The real time, label free approach presented here may facilitate the understanding of mechanisms involves in nanomaterial binding to cell surfaces, in particular identifying the binding partners of bio-nano interface.
- Future studies will be mainly concerned with investigation of binding partners of bio-nano interface.
- **Attana will continue to develop this platform by introducing new hardware and software components.**

Acknowledgement

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