BIO-NANO INTERFACE MODELS APPLIED TO THE INVESTIGATION OF NANOPARTICLES CELL UPTAKE: PROOF OF CONCEPT USING REAL MEMBRANE MODELS

J. Cancino-Bernardi, P.M.P.Lins, V.S. Marangoni, V. Zucolotto

November 7-10, 2016 - Grenoble, France
Potencial applications of nanomaterials in medicine

- Drug delivery
- Theranostics agents - detection and treatment
- Images
- Photothermal and photodynamic therapies

*Adapted with permission from Farokhzad et al. ACSNano v 3, 20 Copyright 2009 American Chemical Society.
However there are potential toxicology effects...

“nanotoxicology would make an important contribution to the development of a sustainable and safe nanotechnology”.

Donaldson et al. (2004)

Aiming to elucidate the mechanisms involved with nanotoxicology aspects!

Motivation

- Complexity of mechanisms involved in the bio-nano interface;
- Molecular level investigation.

✓ Biophysics interaction of nanomaterial in real cell membranes
✓ Using cellular membranes from cancer and health cells
✓ Langmuir and surface techniques
✓ Associate with in vitro studies
Methodology used

Nanoparticles characterization

Physical-chemical characterization of AuNP-PAH, AuNR-PAH and AuNR-PEG
UV-Vis, DLS, zeta potential

Cell membrane extraction and characterization

Extraction of FC3-H and HTC cells membranes and characterization of the lipids and proteins extracted

Membrane models

Reconstitution of extracted cell membranes on subphase with and without nanoparticles

Comparative studies

In vitro studies and ITC interactions to better understand
Membrane extraction and membrane model

- Molecules to come closer together to form an ordered monolayer.
- Simple model systems to investigate the physicochemical properties of biological membranes.

Homogenized cell + lysis buffer → Centrifuge 300g - 10min 4°C → Pellet = cells
→ Centrifuge 10,000g - 20min 4°C → Pellet = dendritic cells
→ Ultracentrifuge 120,000g 2h 4°C → Pellet = membrane
→ Wash and ultracentrifuge 120,000g 2h 4°C → Pellet = membrane

Journal of Proteome Research 2009, 8, 3078-3090
Membrane characterization

**FC3-H**
3% of triglycerides (TAG),
11% of free fatty acids (FFA),
15% of free aliphatic alcohol (ALC),
2% sterol (ST),
6% of mobile polar lipids in ketone (AMPL)
62% of phospholipids (PL)

**HTC**
16% free aliphatic alcohol,
10% mobile polar lipids in ketone
74% of phospholipids.

<table>
<thead>
<tr>
<th>Membrane Protein</th>
<th>Zeta Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC3-H</td>
<td>-10.9 mV</td>
</tr>
<tr>
<td>HTC</td>
<td>-12.9 mV</td>
</tr>
<tr>
<td>3th standard</td>
<td></td>
</tr>
<tr>
<td>FC3H</td>
<td></td>
</tr>
<tr>
<td>HTC</td>
<td></td>
</tr>
</tbody>
</table>

[membrane protein]
Membrane Models

How looks FC3-H and HTC membrane cells at the subphase...

What is important to know?
Has morphology influence the uptake process in FC3-H and HTC membrane cells at the subphase?

**Membrane Models**

+ AuNR-PAH and AuNP-PAH

**Types of interaction**

**Results and Discussion**

jcancino@ursa.ifsc.usp.br
... and charge influences?

Membrane Models

+ AuNR-PEG

Types of interaction

Results and Discussion

jcancino@ursa.ifsc.usp.br
Membrane model vs in vitro results

FC3-H
- uptake of nanoparticles
- no inhibition to adhesion action
- vesicles formation = uptake

HTC
- uptake of nanoparticles
- high inhibition of adhesion
- incorporation through the monolayer

Composition of the cell membrane had the major influence

Cell adhesion results
Summary

✓ The extracted membranes from FC3-H and HTC cells were characterized and they revealed high differences in their composition.

✓ The reconstitution of the membranes on the subphase showed that HTC formed more stable monolayers compared to FC3-H.

✓ An expansion or decrease of the molecular area were indicatives that NP or NR affect packing of the lipids.

✓ The models revealed that not morphology but charge is mandatory in uptake.

✓ FC3-H cell allows NPs uptake through the cell more easily, while HTC probably adsorbs NPs on the cell surface before uptake.

✓ Such investigation may be of great importance to understanding toxicity of nanomaterials at molecular level.
Acknowledgements

Thank you for your attention!

Contacts:
jcancino@ursa.ifsc.usp.br
jcancinobernardii@gmail.com