Influence of biomolecular corona on nanoparticle internalization, in multiple cell lines, using size kit carboxylic polystyrene microspheres

Tiago Alexandre dos Santos (3411)

Centre for BioNano Interactions
University College Dublin

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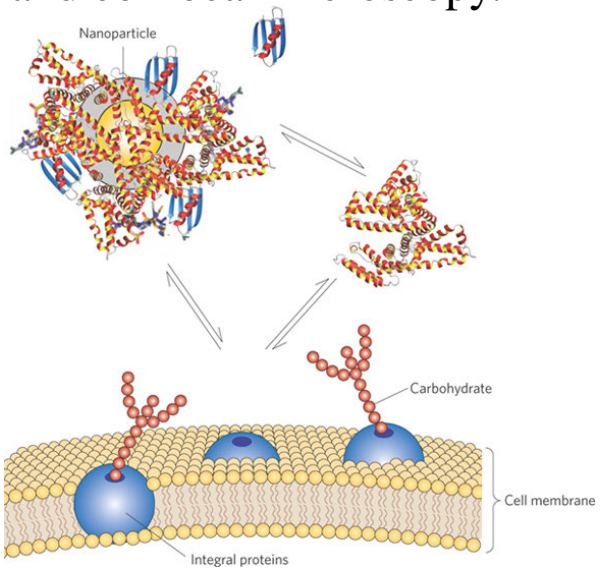
Introduction

Purpose of the visit

The aim of this project was to compare the levels of internalization and final sub-localization, between fluorescent carboxylic- polystyrene nanoparticles of different sizes (40nm, 200nm) with different protein “coronas”, in a panel of cells such as HeLa, A549, 1321N1 and RAW 264.7. In this context, samples have to be prepared by dispersing the nanoparticles either in serum-free medium, or in cMEM (normal medium with addition of foetal bovine serum), leading to different nanoparticle-protein complexes composition, and the main techniques to be used include cell culture, flow cytometry and confocal microscopy.



Introduction

The "protein corona"

Once nanoparticles are dispersed in a biological fluid (plasma, or otherwise) they act as a scaffold for biomolecules such as proteins, lipids etc. which they rapidly adsorb to their "naked" surface, conferring a biological identity to the nanoparticle, leading to the so called protein "corona". During the nanoparticle internalization process into the cell, the adsorbed protein layer "corona" is what the cell will "see", therefore nanoparticle-protein complex composition and structure, etc. will influence not only cell uptake and trafficking, but also particle biodistribution within the cell.

Depending on the cellular morphology and its tissue origin, nanoparticles can be internalized by cells using different mechanisms, and this project aims to elucidate if typical cell surfaces will have great affinity for bare particles than to the protein "corona" layer surrounding the nanoparticles.

The systems investigated

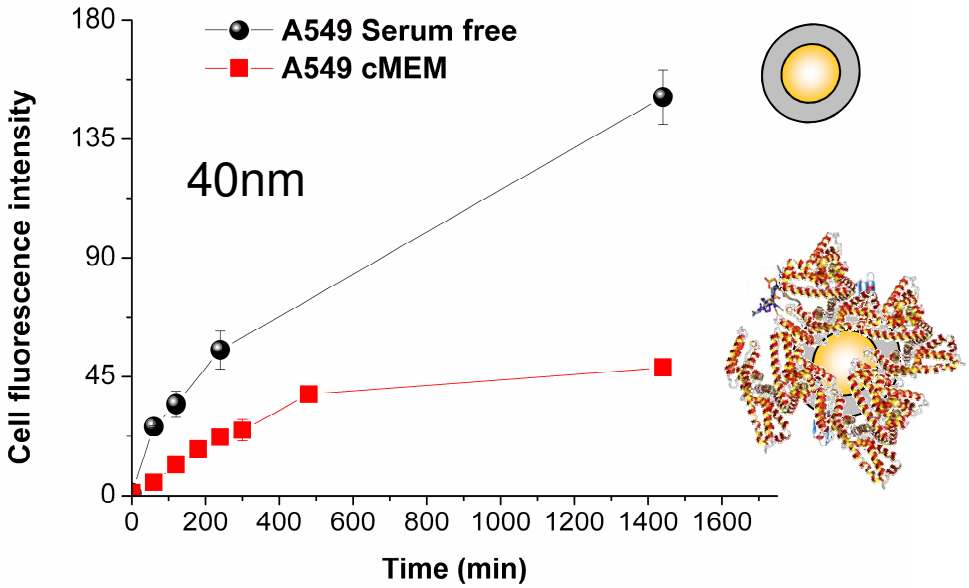
Cell line	Type of cell line
A549	Human lung epithelial carcinoma
HeLa	Human cervical epithelial carcinoma
1321N1	Human astrocytoma
Raw 264.7	Mouse monocyte macrophage

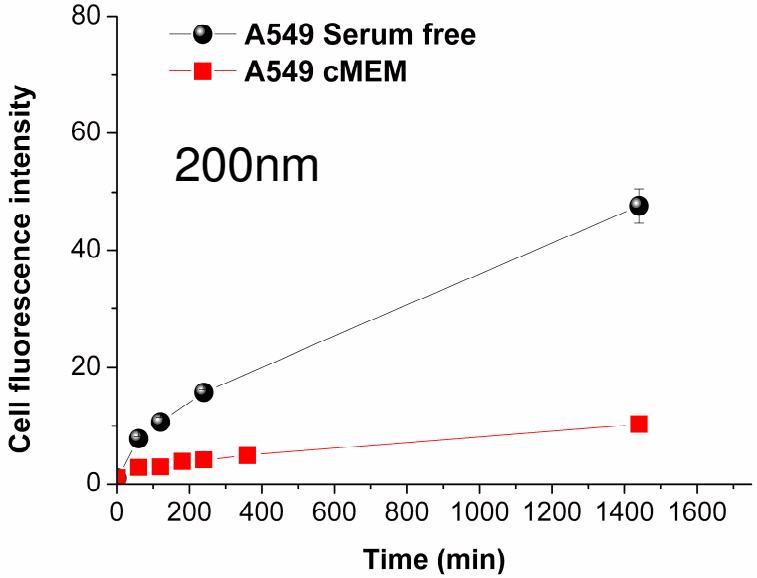
Fluorescent size Kit, carboxylate-modified **polystyrene** microspheres

Size (nm)	DLS size(nm) in water	
40	68	72
	75	
	73	
200	275	270
	252	
	284	

Nanoparticles were dispersed either in serum-free medium, or in cMEM (normal medium with addition of 10% foetal bovine serum)

Main Results obtained

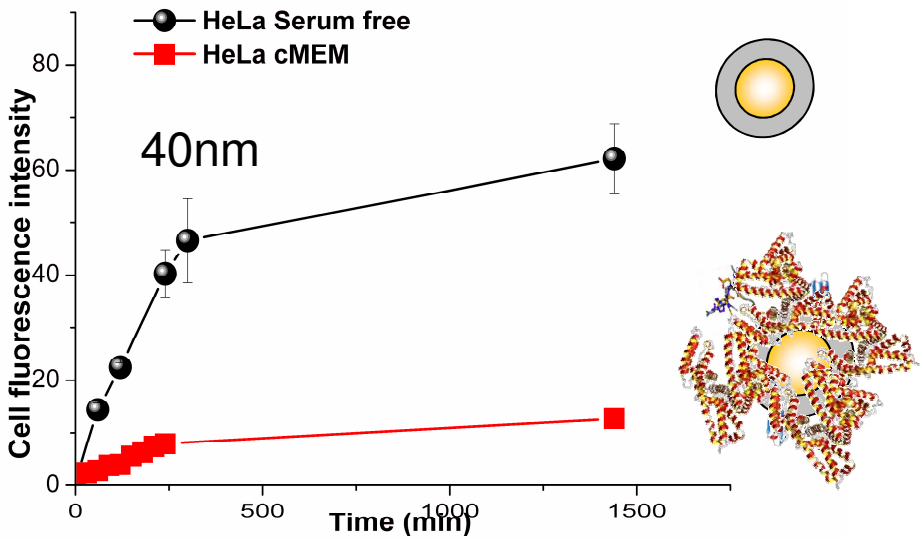


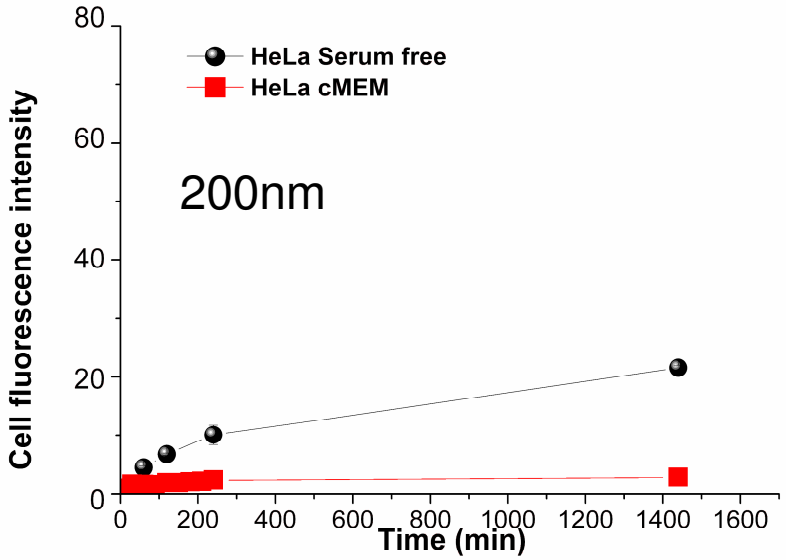


- Curves shows that **protein adsorption** on the surface of polystyrene nanoparticles (40nm) **decreases the subsequent uptake** of the nanoparticles by A549 cells

- The decrease on the nanoparticle uptake in A549 cells, due to protein absortion is size independent, i.e. we observe the same behaviour described above for smaller particles (40nm)

Main Results obtained





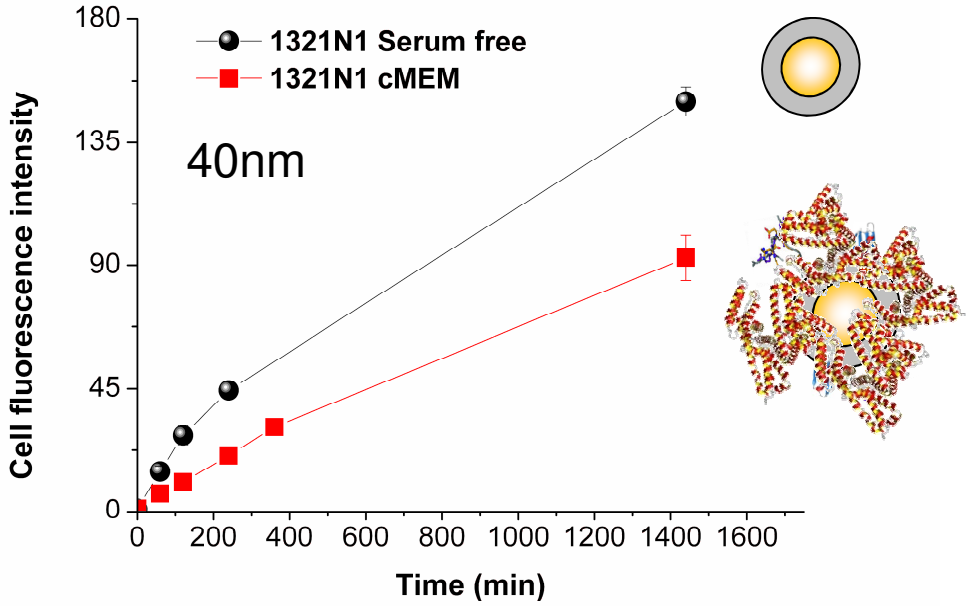
- Same decrease in particles (40nm and 200nm) uptake observed in A549 cells, after their surface protein absortion, this time in HeLa cells

- This phenomenon may be due to hydrophobic effects.

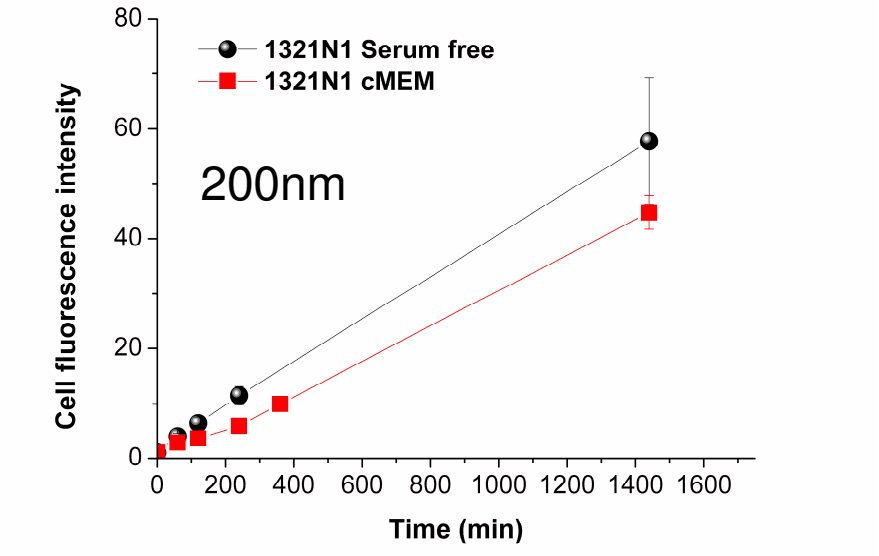
- In the cell uptake process, high surface hydrophobicity of bare nanoparticles will cause them to adhere to the already hydrophobic plasma membrane.

- In the presence of serum, adsorption of proteins screens the non-specific hydrophobic binding of the nanoparticles to the cell surface leading to a decrease of the surface hydrophobicity and a consequently lower, but more specific uptake of the nanoparticles via cellular receptors.

Main Results obtained

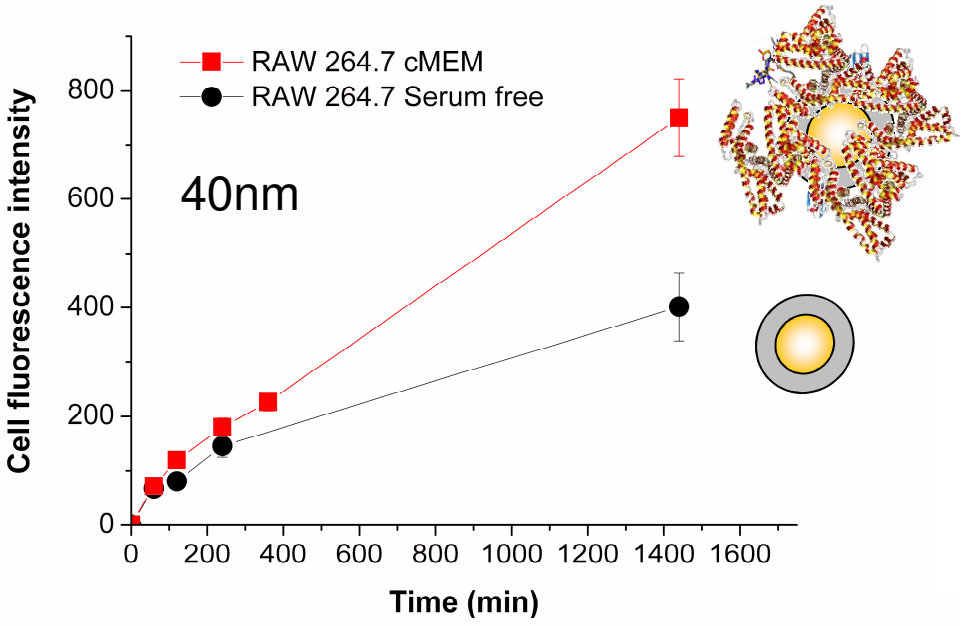


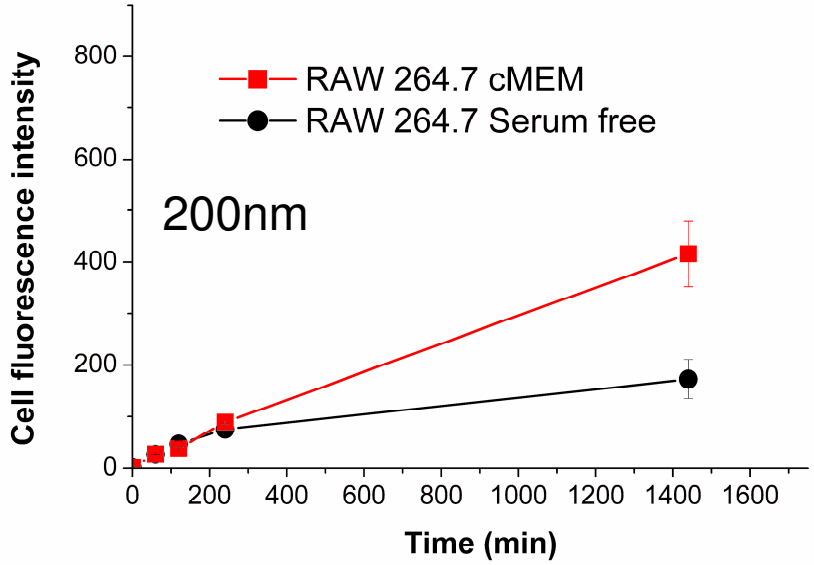
- Again similar behaviour, with curves showing that **protein adsorption** on the surface of polystyrene NPs (40nm) **decreases the subsequent uptake** of the nanoparticles this time in 1321N1 cells



- Also for 1321N1 cells, the decrease on the nanoparticle uptake, due to protein absortion, is size independent.

Main Results obtained





- Different results were obtained when same nanoparticles (40nm and 200nm) were dispersed in the two different media (cMEM and serum free MEM), were exposed to RAW 264.7 cells, a phagocytic cell type

- When nanoparticles are surrounded by a “corona” of proteins the level of cellular uptake was higher when compared with similar bare particles in absence of protein serum

- When nanoparticles are dispersed in the biological medium, they will bind specific proteins that are able to activate the cellular machinery in these phagocytic cells, increasing their internalization

Conclusions:

HeLa, A549 and 1321N1 (non-phagocytic cell type), showed that nanoparticle uptake is higher in serum free conditions, for all sizes studied.

High surface hydrophobicity of bare nanoparticles will increase the adherence to the cell membrane, increasing the uptake

Only in the case of the macrophages in serum free conditions a higher uptake efficiency is observed for nanoparticles covered with proteins on their surface, as opposed to the same material in serum free conditions.

The specificity of some protein on the nanoparticle surface, are able to activate the cellular machinery in these phagocytic cells, when nanoparticles are dispersed in the biological medium.

Future work

Since a possible explanation for the quite distinct behaviour observed in macrophage cells can be due to the fact that when nanoparticles are dispersed in the biological medium, they will bind specific proteins, it would be interesting to recovery the nanoparticles following uptake, and identify their corona, to point out eventual proteins responsible for their internalization.