Effects of drug inhibitors of endocytosis on uptake of carboxylic polystyrene microspheres in multiple cell lines

Tiago Alexandre dos Santos (2877)

Centre for BioNanoInteractions
University College Dublin

Final Scientific Report

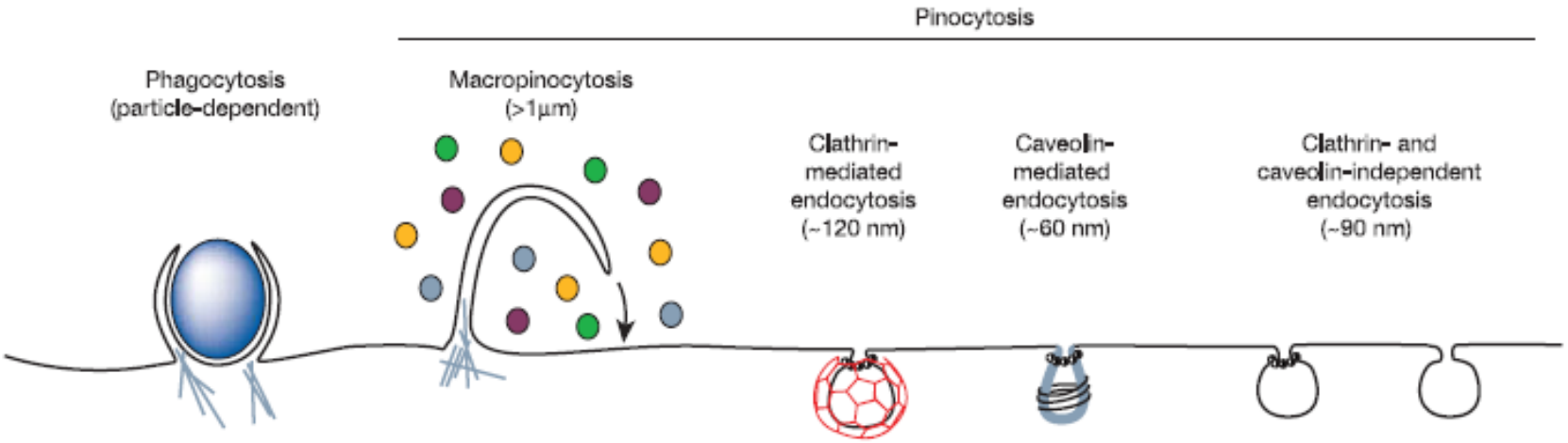
EUROPEAN SCIENCE FOUNDATION

UCD DUBLIN

Introduction

Purpose of the visit

The aim of this project was to test a series of drugs inhibitors of each of the multiple mechanisms of endocytosis, in order to determine their effects on the uptake of fluorescent carboxylic-polystyrene nanoparticles of different sizes (40nm, 200nm), in different cell lines such as HeLa, A549, Raw 264.7, 1321N1, HCMEC-D3. Depending on the cell morphology and their tissue origin, nanoparticles can be internalized by cells using the different mechanisms of endocytosis described in the figure below. The aim was a systematic evaluation of the uptake mechanisms utilised by the differently sized nanoparticles in the different cell lines.



Introduction

Endocytosis Inhibitors used

Cytochalasin A - have the ability to bind to actin filaments and block polymerization and the elongation of actin. As a result of the inhibition of actin polymerization, cytochalasins can change cellular morphology, inhibit cellular processes **(5µg/ml)**

Chlorpromazine, inhibits clathrin-mediated endocytosis. **(10µg/ml)**

Nocodazole, interferes with the polymerization of microtubules. Microtubules are one type of fibre which constitutes the cytoskeleton, and the dynamic microtubule network has several important roles in the cell, including vesicular transport and in cytokinesis **(20µM)**

Genistein, a specific inhibitor of protein tyrosine kinase, consequently for lipid rafts and caveolin mediated endocytosis **(200µM)**

Each drug targets a different protein at the cell surface or involved in one of the receptor-mediated uptake processes. The effect of removing these proteins on nanoparticle uptake was investigated systematically.

The systems investigated

Cell line	Type of cell line
A549	Human lung epithelial carcinoma
HeLa	Human cervical epithelial carcinoma
1321N1	Human astrocytoma
HCMEC D3	Human brain endothelial
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	

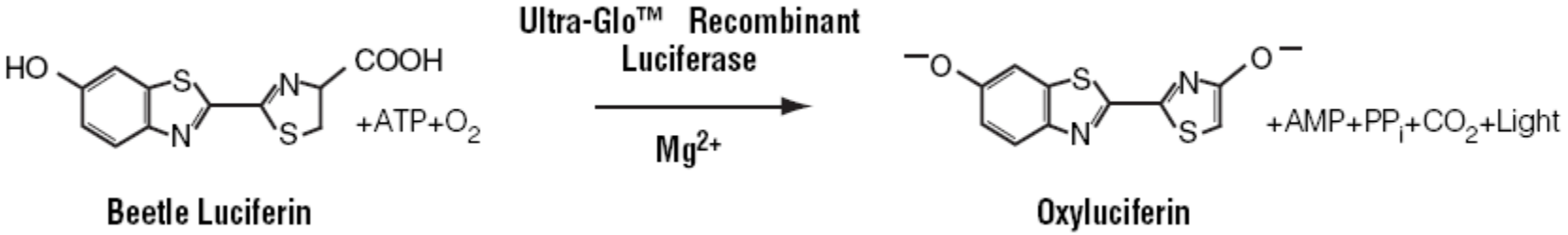
Uptake: 30min pre-incubation of cells with one of the inhibitors followed by 2h uptake (continuous) of the NPs + the chosen inhibitor.

Main Results obtained

Cell viability determined via ATP and PI assays

These assays were performed for each cell line adn each inhibitor, to ensure that cells remained viabile and that uptake condistions were similar in each experiment.

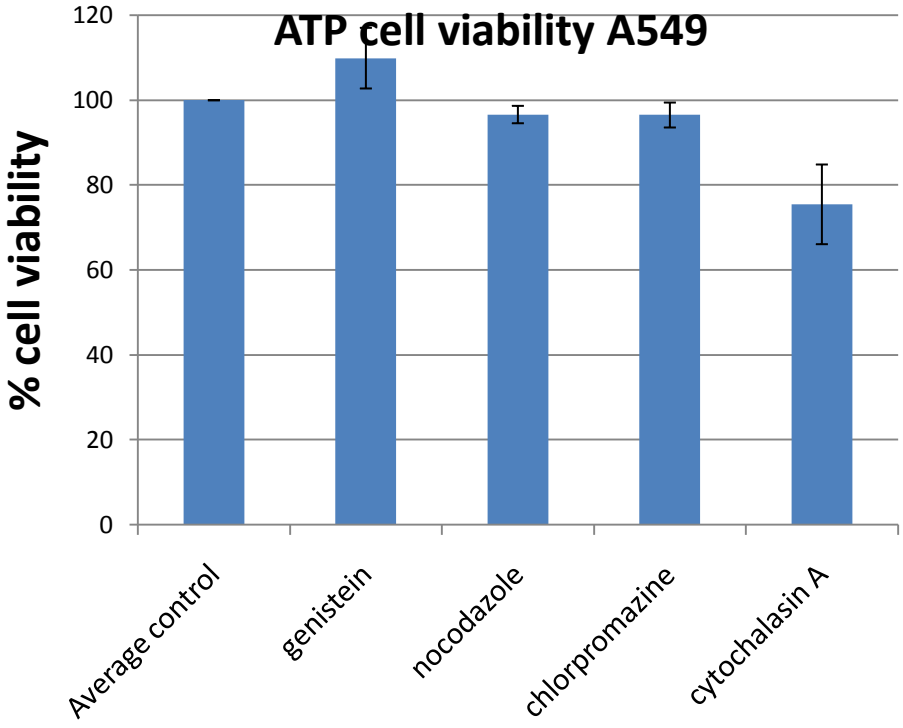
ATP assay: CellTiter-Glo® Luminescent Cell Viability Assay is a method to determine the **number of viable cells** in culture based on **quantitation of the ATP** present, which signals **the presence of metabolically active cells.**

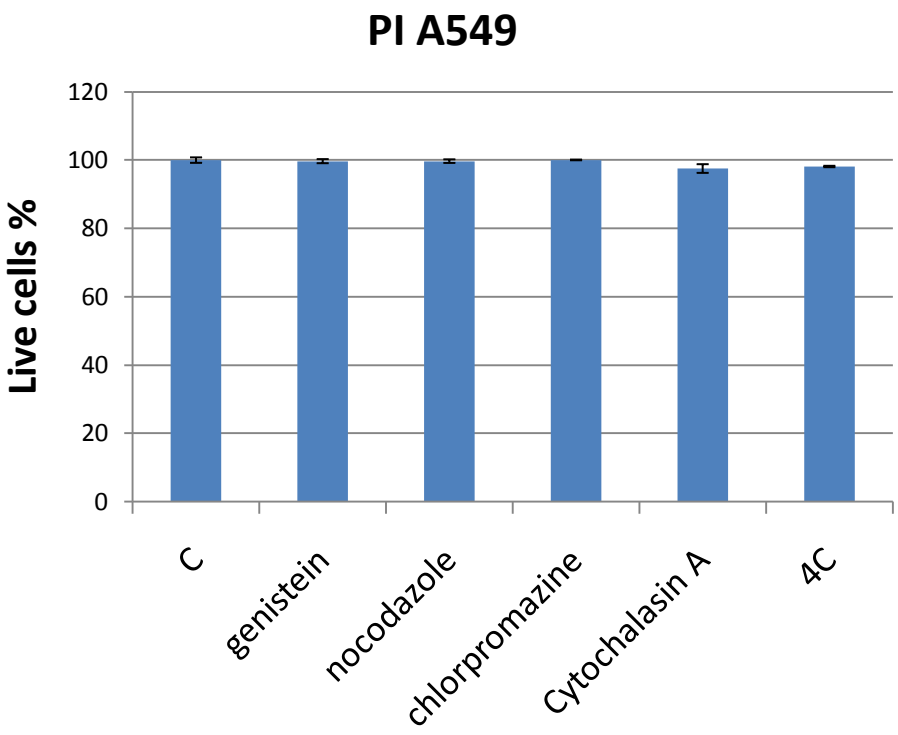


Beetle Luciferin

Oxyluciferin

Propidium iodide (PI) was used as a reporter for cell damage in response to the presence of the nanoparticles, as this dye only penetrates **damaged cellular membranes.**.





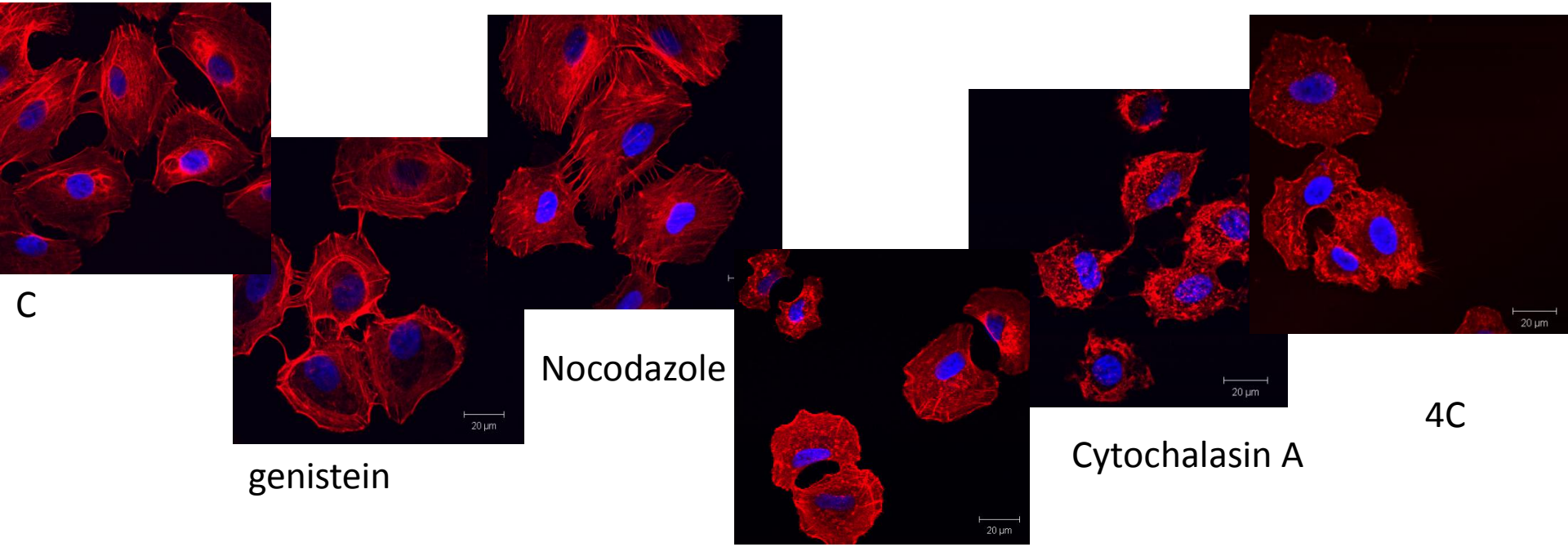
Time of drugs exposure : 2 h

Cell line used: Above shows one example with A549 cell line, but these assays were performed for all other cell lines.

Results confirm **80-100%** of cell viability, for all cell lines in study in the presence of each of the drugs tested. Thus, we determined that we could use each of the inhibitors to assess the effect of inhibition of the uptake pathways on the uptake of carboxylate-modified polystyrene nanoparticles..

Checking F-actin structure after inhibitor exposure

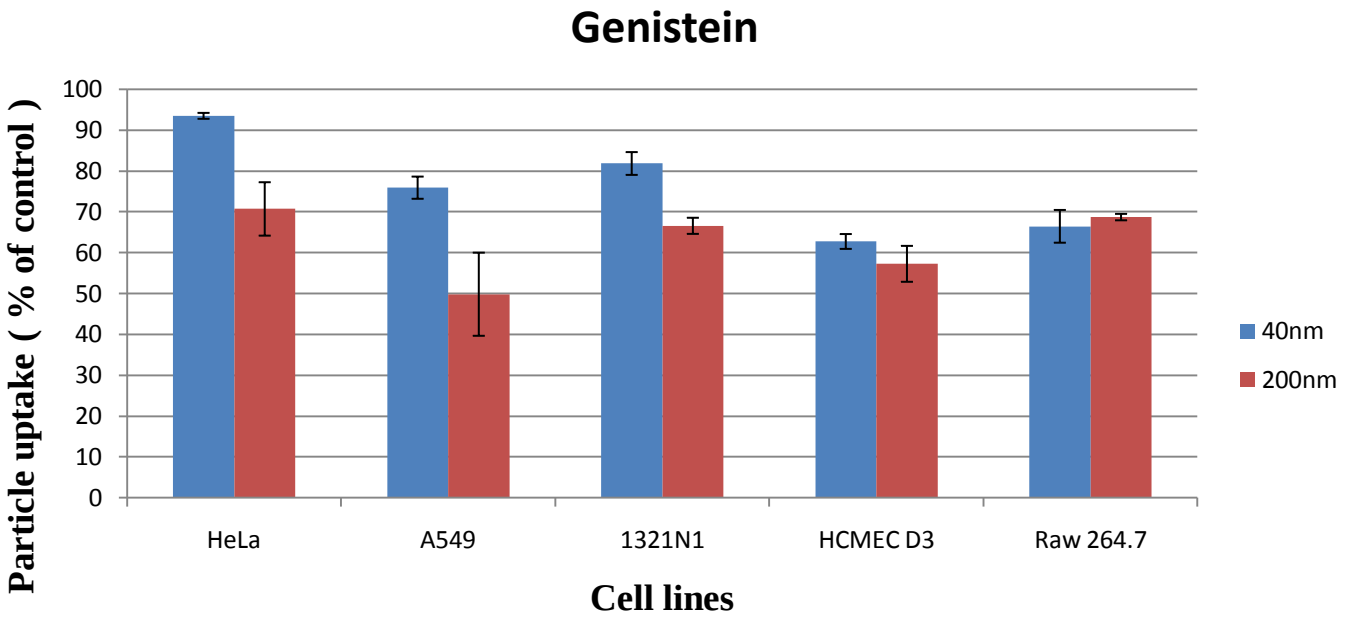
A549



F-actin was stain with Texas-red phalloidin, in order to check the morphology of the cells after treatment with each of teh different inhibitors.

Results confirm that actin fillaments maintain their main structure after 2h of exposure to each of the various inhibitors, with the exception of cytochalasin A, a known actin-disrupting agent. These experiences were performed in all cell lines, showing similar results.

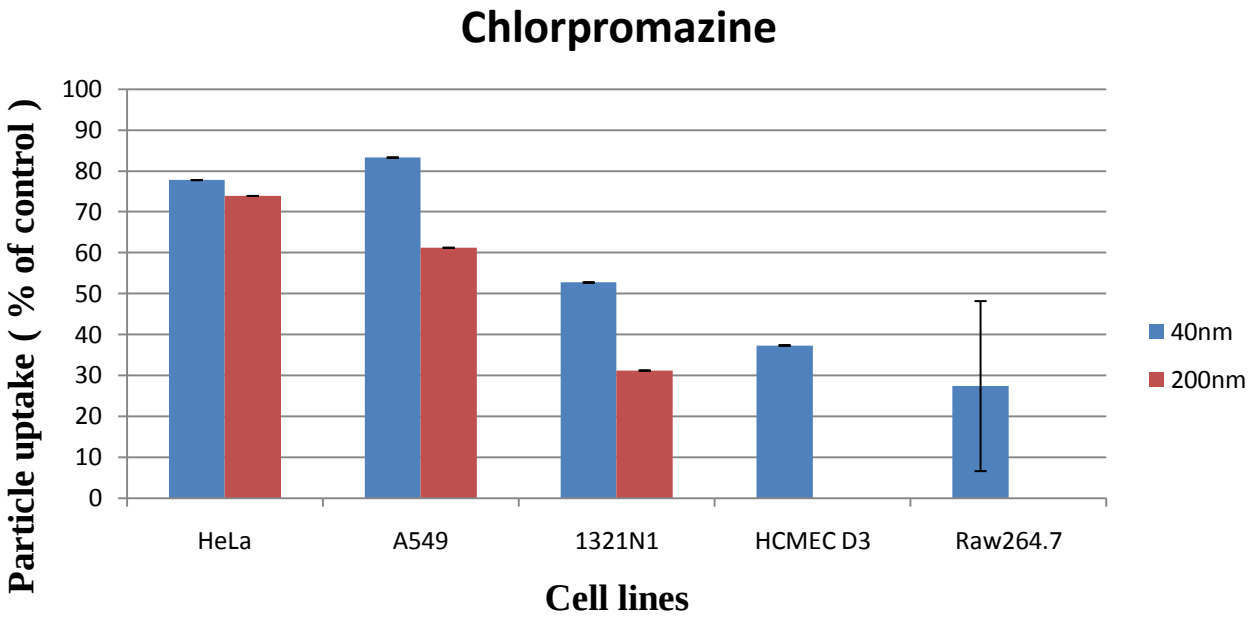
Genistein 200µM



Strong inhibition (50%) of nanoparticle internalization in A549 cells, especially for 200nm nanoparticles.

The trend across all the different cell lines was a higher inhibition for bigger particles (200nm), when compared with 40nm, especially for HeLa, A549 and 1321N1 cells.

Chlorpromazine 10µg/ml

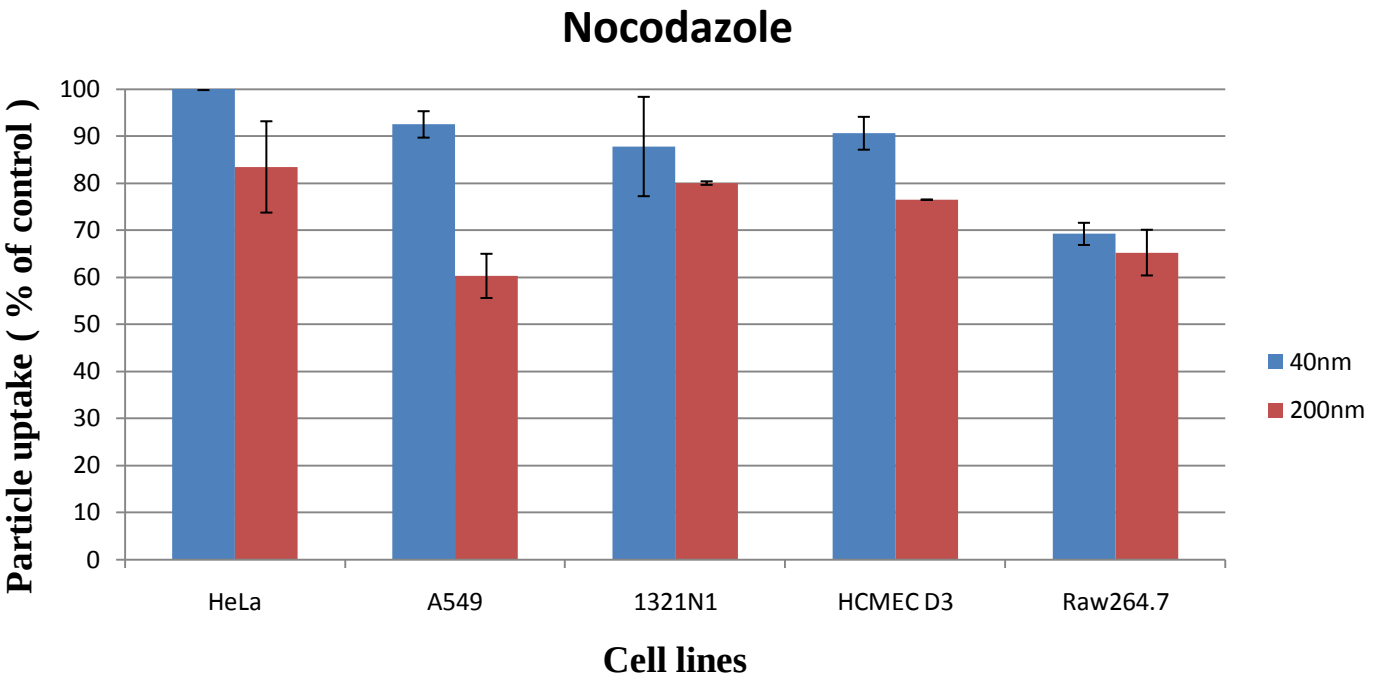


Higher inhibition for both size particles in 1321N1 cell line than for HeLa or A549 cells. A significant (70%) decrease for 200nm, and a 50% inhibition for 40nm particles was observed in the 1321N1 cells.

No cells were found after exposure of chlorpromazine and 200nm nanoparticles in HcMEC D3 and Raw264.7. More experiments needs to be performed in order to clarify why this happened.

The trend in the level of inhibition of both particles was found for HeLa, A549 and 1321N1

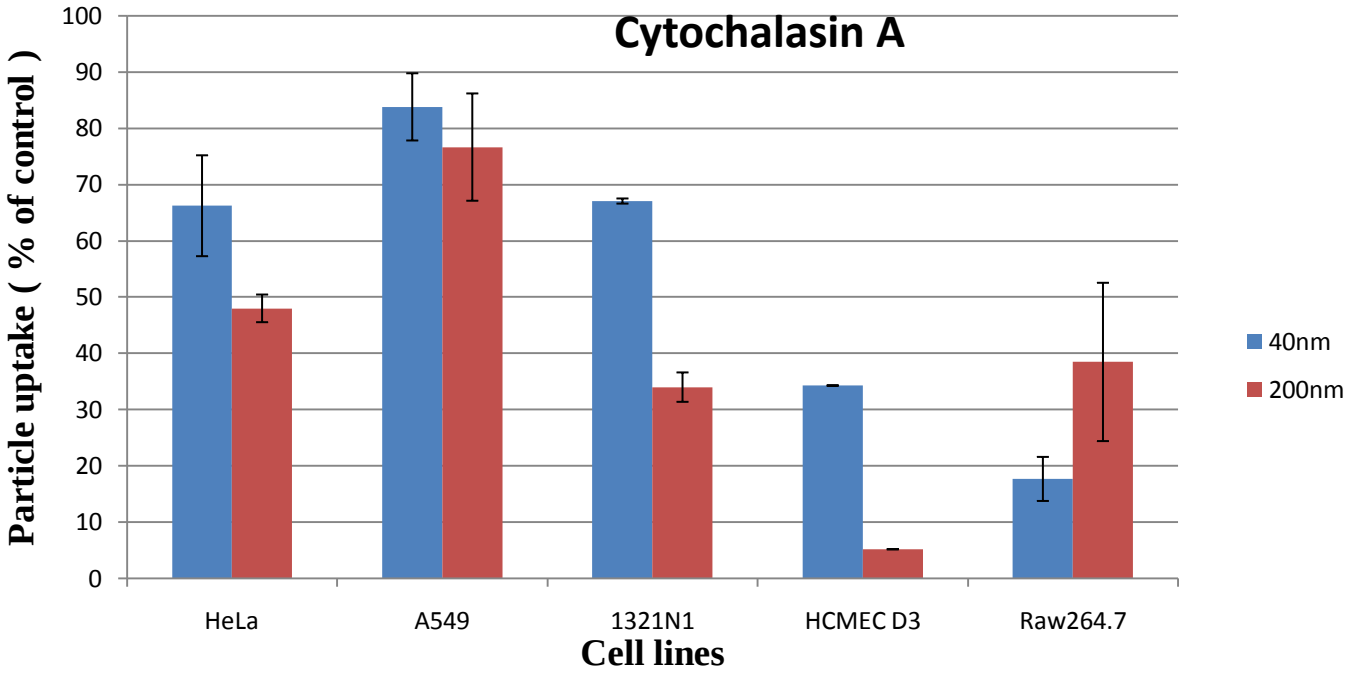
Nocodazole 20µM



Inhibits mainly endocytosis of bigger particles (>200nm), with a higher inhibition for A549 cell line, of about 40% comapred to the control case in the absence of inhibitor.

For smaller particles (40nm) inhibition was quite small (5-10%), in the case of A549 and 1321N1 cells, or even equal to the control for HeLa cell line.

Cytochalasin A 5µg/ml



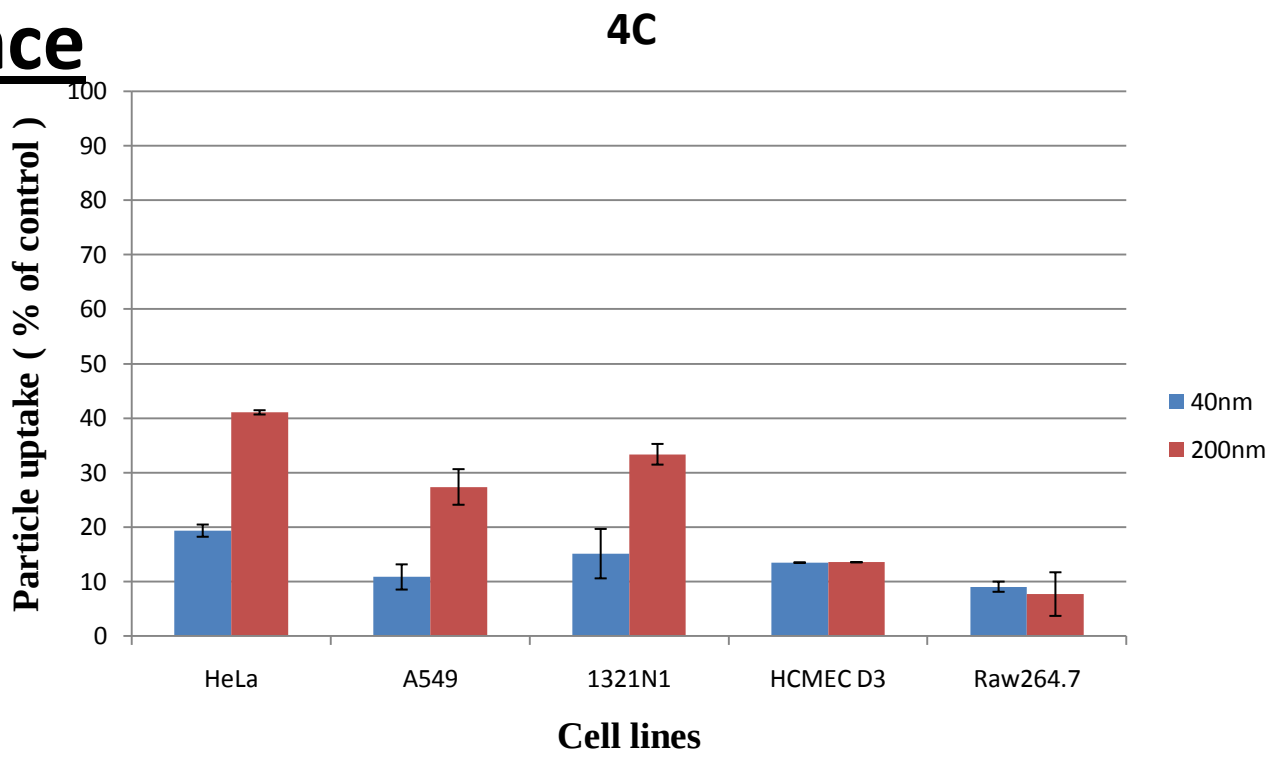
Uptake of 40nm particles is inhibited by about 30% in the presence of cytochalasin A, in the case of HeLa and 1321N1 cells, by 70% in HcMEC D3 cells, and only by about 10% in the case of A549 cells.

Stronger inhibition of uptake of 200nm nanoparticles was observed in the presence of Cytochalasin A: uptake was reduced by 50% for HeLa cells, by 65% for 1321N1 cells, and by 95% for HcMEC D3 cells, indicating a possible change of uptake pathway for bigger particles

Similar observations were made when comparing uptake in the presence of nocodazole, where again a stronger dependency of uptake of 200nm nanoparticles on f-actin and microtubules than for 40nm particles.

Energy dependence

4 ^{o}C



Endocytosis mechanism is highly energy dependent.

Smaller particles (40nm) showed a stronger inhibition when compared with 200nm, especially for HeLa, A549 and 1321N1, indicating that a different mechanism of endocytosis can be used for different sizes, requiring different levels of energy

Conclusions:

All cell lines showed that endocytic pathways are energy dependent, as NP uptake was reduced dramatically in the absence of cellular energy.

Bigger particles (200nm) seems to require less energy to be internalized, in cell lines such as HeLa, A549, 1321N1, which are not specalised for phagocytosis (uptake of larger particles).

An higher inhibition of uptake of bigger particles (200nm) is observed using inhibitors of chlatrin and caveolae dediated endocytosis, suggesting these are important uptake mechanisms for these particles.

HeLa cells seem to use preferentialy a **chlatrin dependent endocytosis mechanism**, specially for 40nm particles. For 200nm either chlatrin and caveolae are required. Actin filaments are also required.

Uptake of 200nm particles by **A549** cells is **both chlatrin and caveolae** dependent (30% and 50% of control inhibition). A similar observation was made for 40nm, although the inhibition was not so clear (20%). Actin filaments do not seem to be required so much (10% inhibition)

Particles (both 40nm and 200nm) in **1321N1** cell line, seems to prefer a **Chlatrin dependent Mediated Endocytosis** , rather caveolae dependent ME, specially for bigger particles. Actin filaments are required, specially for bigger particles

Future publications

This work will result in a manuscript which is *in preparation.*

Acknowledgment of the ESF financial support will be included in this publication

Effects of drug inhibitors of endocytosis on uptake of carboxylic polystyrene microspheres in multiple cell lines

Tiago dos Santos, Juan Varela, Anna Salvati, Iseult Lynch, Kenneth A. Dawson**

Centre for BioNano Interactions, School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland