Evaluating the uptake, intracellular fate and functional consequences of hepatocyte exposure to a range of nanoparticles, *in vitro.*

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Declaration

It is hereby declared that this thesis and the research work upon which it is based were conducted by the author, Helinor Johnston.

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Abstract

The liver is recognised as a potential target site for nanoparticle (NP) toxicity, as NPs have been observed to accumulate within this organ subsequent to exposure via injection, inhalation or instillation. The liver's unique structure has to be taken into consideration when evaluating NP toxicity, as a variety of cell types of distinct morphology and function are evident, and potentially affected by NP exposure. Of particular interest are hepatocytes, due to their abundance and importance to the maintenance of normal liver function, and macrophages due to their role in host defence.

The uptake and intracellular fate of fluorescent polystyrene particles (20nm and 200nm) by hepatocytes was evaluated (with exposure times of up to 60 minutes). Within the studies conducted comparisons of the response of primary rat hepatocytes, with C3A and HepG2 hepatocyte cell lines to NP exposure were made in order to investigate whether cell lines are a relevant model of hepatocyte behaviour. It was found that the uptake of particles by the primary hepatocytes, and both cell lines was size and time dependent. Specifically, it appeared that the internalisation of 200nm particles was limited, occurred at later time points (60 minutes), with the majority of particles evident at the cell surface. Polystyrene NPs (20nm) were internalised by cells after a 10 minute exposure time, after which NPs compartmentalised either within and/or between adjacent cells. The nature of the NP 'compartments', and therefore fate of internalised NPs was then investigated to determine if the compartments developed as a consequence of the mechanism of uptake, or due to the attempted elimination of NPs from cells. It was found that NPs were not contained within early endosomes or lysosomes. However it was apparent that polystyrene NPs were eliminated to a limited extent within the bile canaliculi of all cell types, and may accumulate within the mitochondria of cell lines after a 60 minute exposure, which warrants further investigation.

The impact of the PARTICLE_RISK particle panel [consisting of ultrafine carbon black (ufCB), CB, carbon nanotubes (CNTs), C_{60} (carbon fullerene) QD621 (positively charged quantum dots) and QD620 (negatively charged quantum dots)] on hepatocyte function was then determined. It was consistently observed that QD621 and QD620 were able to elicit the greatest extent of

toxicity, evidenced within their ability to deplete cellular GSH, induce cytotoxicity, initiate a pro-inflammatory response (indicated by an increase in IL-8 production) and decrease bile secretion, in the hepatocyte couplet, *in vitro* model. It was observed that the pattern of response was similar within the cell lines and primary cells.

Differentiated monocytic THP-1 cells (to represent the resident liver macrophages, Kupffer cells) were exposed to the PARTICLE_RISK particle panel to obtain conditioned medium (CM) that was exposed to hepatocytes, in order to gain insight into the ability of macrophages to influence NP mediated toxicity to hepatocytes. Firstly, the response of macrophages to particle exposure was considered and it was apparent that the toxicity that was observed within hepatocytes was paralleled within the response of differentiated monocytic cells (THP-1). Accordingly, QD621 were again proven to have the greatest toxic potential, with QD620 able to induce toxicity to a more limited extent. The exposure of hepatocytes to CM potentiated the toxicity observed when cells were exposed to particles alone, so that the pattern of response was comparable, but the extent of toxicity greater, and evident at earlier time points. It was apparent that QDs were able to induce an inflammatory response (characterised by TNFα and IL-8 production) within the liver that was primarily mediated by macrophages.

When considering the results from all experiments it is evident that some of the particles contained within the PARTICLE_RISK panel were more capable of eliciting toxicity within the liver, and that their toxicity can be ranked in the following order: QD621>QD620>CNT=ufCB=C_{60}>CB.

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Supplementary Data DVD

A supplementary data DVD has been provided that contains animations associated with the following figures:

Figure 4.6. Confirming the uptake of 20 or 200nm polystyrene particles by C3A cells using Z stacks

Figure 4.7. The live imaging of fluorescent polystyrene particle uptake (20nm and 200nm) by C3A and HepG2 cells, in the presence and absence of serum

•If the speed of the animations is too fast within the z stacks featured within figure 4.6, then I recommend pausing the movie, and using the 'clip position' tool to move through the different optical slices of the image.

•The z stacks were also converted into 3D projections, which can also be viewed within the supplementary DVD.

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Firstly I would like to thank Prof Vicki Stone for her continued support throughout my PhD. Her guidance has been an integral part of completing my PhD and her knowledge of all things nano, and couplet colleting skills, really are impressive.

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Abbreviations

α	alpha
AFM	atomic force microscopy
ARE	antioxidant response element
ATP	adenosine triphosphate
ß	beta
BSA	bovine serum albumin
BSP	bromosulphophthalein
C_{60}	carbon fullerene
Ca	calcium
$CaCl_2$	calcium chloride
CB	carbon black
CCl_4	carbon tetrachloride
CdSe	cadmium selenide
CdTe	cadmium telluride
CM	conditioned medium
CME	clathrin mediated endocytosis
CNT	carbon nanotube
CO_2	carbon dioxide
COPD	chronic obstructive pulmonary disease
CLF	cholyl lysyl fluorescein
CVA	canalicular vacuole accumulation
CYP450	cytochrome P450
dH_2O	distilled water
DCFH	dichlorofluorescin
DCFH-DA	2,7-dichlorofluorescein diacetate
DLS	dynamic light scattering
DNA	deoxyribonucleic acid
DEP	diesel exhaust particles
DPPC	dipalmitoylphosphatidylcholine
DTT	dithiothreitol
ECV	endosomal carrier vesicle
EDTA	ethylenediaminetetraacetic acid
EEA-1	early endosome antigen 1
ELISA	enzyme linked immunosorbant assay
EM	electron microscopy
ER	endoplasmic reticulum
FCS	fetal calf serum
FFF	field flow fractionation

Abbreviations (continued)

GIT	gastroIntestinal tract
H_2SO_4	sulphuric acid
HBSS	hanks balanced salt solution
HO-1	heme oxygenase
Hr	hour
HPLC-MS	high performance liquid chromatography
HRP	horse radish peroxidise
ICP-MS	inductively coupled plasma mass spectroscopy
i.t	intra-tracheal
i.v.	intravenous
IRHC	isolated rat hepatocyte couplet
IL	interleukin
LCD	liquid crystal display
LAMP-1	lysosome associated protein 1
M	molar
m	metre
MEM	minimum essential medium
mins	minutes
ml	millilitre
mM	millimolar
mRNA	messenger ribonucleic acid
MTT	2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl- 2H-tetrazolium bromide
$MgCl_2$	magnesium chloride
MWCNTS	multi walled carbon nanotubes
MRP2	multridrug resistant protein 2
mm	millimetre
NaCl	sodium chloride
NaOH	sodium hydroxide
Na/K ATPase	sodium/potassium pump
NADH	β-nicotinamide adenine dinucleotide
NF- kß	nuclear factor kappa beta
NADPH	nicotinamide adenine dinucleotide phosphate
NEM	N-Ethylmaleimide
nm	nanometre
NP	nanoparticle
NO	nitric oxide
OPT	*o*-phthaldialdehyde
PAH	polycyclic aromatic hydrocarbons

Abbreviations (continued)

PBS	phosphate buffered saline
PEG	polyethylene glycol
Pen/Strep	penicillin streptomycin
PM	plasma membrane
PMA	phorbyl myristate acetate
POC-R	perfusion, open and closed cultivation
P-gp	p-glycoprotein
QD	quantum dot
RBC	red blood cell
RES	reticuloendothelial system
ROS	reactive oxygen species
SA	surface area
SARs	structure activity relationships
SEM	scanning electron microscopy
Strep-PE	streptavadin phycoerythrin
SWCNTS	single walled carbon nanotubes
TEM	transmission electron microscopy
TNF	tumour necrosis factor
TiO_2	titanium dioxide
UV	ultraviolet
UVR	ultraviolet radiation
ufCB	ultrafine carbon black
w/o	without
ZnS	zinc sulphide
4HNE	4 hydroxynoneal
8-O-H-G	8hydroxyguanosine
µg	microgram
µl	microlitre

Publications

- Stone V, Johnston H and Clift MJD (2007). Air Pollution, ultrafine and nanoparticle mediated toxicology: cellular and molecular interactions. *IEEE Trans Nanobioscience* **6**; 331-340

- I was also involved in the DEFRA funded CELL PEN report (2008) *In Press*. This report investigated the mechanisms of nanoparticle translocation across cell membranes.