GENE EXPRESSION RESPONSES IN THE THREE-SPINED STICKLEBACK *(Gasterosteus aculeatus)* FOLLOWING SHORT TERM EXPOSURE TO SILVER QUANTUM DOTS

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Introduction

The use of nanomaterials within industrial, medical and remediation processes is likely to increase over the next decade and as a consequence the potential for environmental exposure to engineered nanoparticles (ENPs) will increase. While the use of ENPs represents significant advances in material sciences, adverse biological effects following exposure to ENPs such as carbon nanotubes, metal oxides and Quantum Dots (QDs) have been reported. Much of work on biological impacts of ENP exposure has focussed on air-born exposure and there remains a paucity of information on their effects within the aquatic environment.

Quantum dots (QD) are nanocrystals with a metallic core, surrounded by an inorganic shell. QDs have a variety of applications in bio-imaging, photovoltaics and lighting. Recently, the antibacterial properties of silver nanoparticles has driven their inclusion into a variety of commercial products. The rapid growth of this industry has prompted concern over the impact on human health and the environment.

Using a stickleback cDNA microarray this study investigated changes in transcript levels of liver and brain tissue following short term exposure to methyl polyethylene glycol (MPEG)-coated silver QDs. Initial RT-qPCR markers were then developed in an attempt to develop quantitative tools for assessing the biological impacts of silver nanoparticle exposure.

Materials and Methods

- **Sterically stabilised silver nanoparticles (Ø =13±7nm) were prepared by reducing silver nitrate in the presence of MPEG to produce silver QDs capped with MPEG (nAg) (Figure 1).**
- **3-spined sticklebacks exposed for 48h under static conditions to nAg (5, 50 & 500μgL^{-1}; Ø >500nm) or MPEG (500μgL^{-1}).**
- **Gene expression of liver and brain tissue investigated using stickleback cDNA microarray**
- **SYBR green based qPCR was performed with primers based on EST sequence and used for microarray validation**

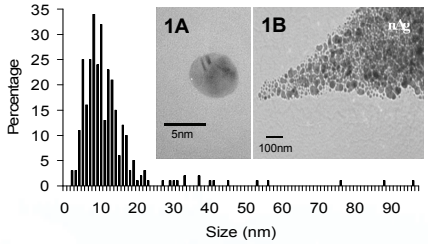

Figure 1: Size distribution of MPEG capped silver nanoparticles (1A) as measured by TEM (1B)

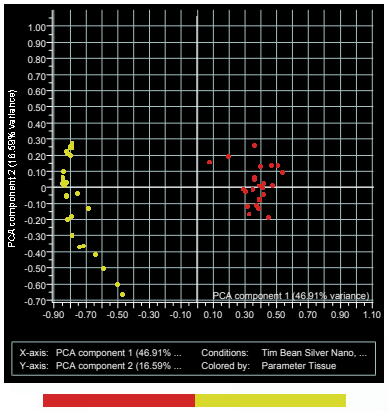

Figure 2: Principal Component Analysis (PCA) of gene expression in liver and brain tissue

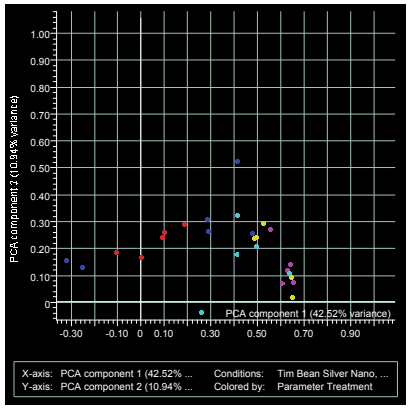

Figure 3: PCA of gene expression in brain

Nominal	T=0	T=48
nAg 5 μgL^{-1}	< LOD	< LOD
nAg 50 μgL^{-1}	< LOD	< LOD
nAg 500 μgL^{-1}	44 ± 7	52± 7
Bulk Ag 500 μgL^{-1}	< LOD	< LOD
MPEG 500 μgL^{-1}	< LOD	< LOD
Control	< LOD	< LOD

Table 1: Mean (n=3) silver concentrations (μgL^{-1}) of test solutions at t=0 and t=48h, measured by ICP-AES with standard deviations. Limit of Detection (LOD) = 10 μgL^{-1}

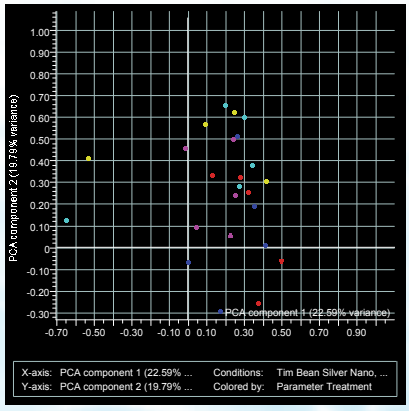

Figure 4: PCA of gene expression in liver

Annotated ESTs with differential expression	nAg 5 μg/L	nAg 50 μg/L	nAg 500 μg/L	Ag
Amyloid beta (A4) precursor protein-binding	0.8	3.2	5.3	4.8
Trafficking protein particle complex subunit 6	0.6	2.0	4.7	4.4
Ornithine decarboxylase antizyme inhibitor	0.8	2.3	4.5	3.7
Calpain-1 catalytic subunit	0.7	2.0	4.5	3.6
Vimentin A1	1.2	2.6	4.2	3.5
Microsomal triglyceride transfer protein large subunit precursor	1.1	3.3	4.0	3.2
Phosphorylase kinase, gamma 1	0.9	2.4	4.0	3.0
Atrophin-1	0.7	2.7	4.0	3.6
FUN14 domain containing	0.9	2.6	3.9	3.7
Proteasome alpha 2	1.0	2.1	3.9	3.0
Mitochondrial import inner membrane translocase subunit Tim13	0.8	2.3	3.9	3.6
Hprt1 protein	1.0	2.1	3.9	3.4
Protein tyrosine phosphatase, receptor type, D (PTPRD)	1.0	2.6	3.8	3.2
Clathrin light polypeptide A	0.9	2.2	3.8	3.6
Breakpoint cluster region	0.7	3.4	3.7	3.6

Table 2: Annotated expressed sequence tags (ESTs) exhibiting highest fold change in expression in stickleback brain following exposure to nAg or bulk silver (500ugL^{-1}) relative to MPEG control. Differences determined by ANOVA based on a False Detection Rate (FDR) of <0.05. Fold changes grouped: <1x green; 1-2x pale blue; 2-3x yellow; 3-4x light orange; 4-5x dark orange; >5x red.

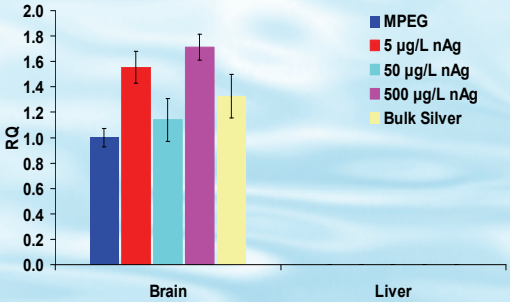

Figure 5: qPCR of vimentin expression relative to MPEG presented with standard error (n=3). Vimentin is a structural protein responsible for maintaining cell shape, cytoplasmic integrity & stabilising cytoskeletal interactions. Transcript not detected in liver.

Results and Discussion

- **Measured concentrations were clearly below nominal (Table 1)**
- **PCA of micro-array data identified tissue specific gene expression (Figure 2).**
- **Initial analysis indicated a cohort of indicator genes (Table 2) displaying dose-dependent expression in brain (Figure 3) but not liver tissue (Figure 4) following exposure to nAg.**
- **Responses in brain to 500μgL^{-1} nAg similar to those elicited by 500μgL^{-1} bulk silver (Figure 3).**
- **Affected genes appear to be involved in functions linked to neural damage and cytoskeletal formation (Table 2).**
- **Initial development of qPCR markers for genes identified by micro-array as responsive to silver exposure e.g. vimentin (Figure 5) do not support a dose-dependent response.**

Conclusions

- **Gene expression changes in the liver and brain appear representative of exposure to silver (regardless of bulk or nanoparticles form) and are primarily associated with neural damage and cytoskeletal formation.**
- **The disparity between nominal and measured silver concentrations prevents identification of the causative factor producing the observed biological effects and merits further investigation.**

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