Taylor & Francis
Taylor & Francis Group

Toxicology Mechanisms and Methods

ISSN: 1537-6516 (Print) 1537-6524 (Online) Journal homepage: http://www.tandfonline.com/loi/itxm20

Toxicity of silver nanoparticles, multiwalled carbon nanotubes and dendrimers assessed with multicellular organism C. elegans

Marta Walczyńska, Witold Jakubowski, Tomasz Wasiak, Kinga Kądzioła, Nina Bartoszek, Sylwia Kotarba, Małgorzata Siatkowska, Piotr Komorowski & Bogdan Walkowiak

To cite this article: Marta Walczyńska, Witold Jakubowski, Tomasz Wasiak, Kinga Kądzioła, Nina Bartoszek, Sylwia Kotarba, Małgorzata Siatkowska, Piotr Komorowski & Bogdan Walkowiak (2018): Toxicity of silver nanoparticles, multiwalled carbon nanotubes and dendrimers assessed with multicellular organism C. elegans, Toxicology Mechanisms and Methods, DOI: 10.1080/15376516.2018.1449277

To link to this article: https://doi.org/10.1080/15376516.2018.1449277

Accepted author version posted online: 02 Apr 2018.

Toxicity of silver nanoparticles, multiwalled carbon nanotubes and dendrimers assessed with multicellular organism *C. elegans*

Marta Walczyńska[1], Witold Jakubowski[1], Tomasz Wasiak[2], Kinga Kądzioła[2], Nina Bartoszek[2], Sylwia Kotarba[2], Małgorzata Siatkowska[1,2], Piotr Komorowski[1,2], Bogdan Walkowiak[1,2],

[1]Division of Biophysics, Institute of Materials Science and Engineering, Lodz University of Technology, Stefanowskiego 1/15, 90-924 Lodz, Poland

[2]Laboratory of Molecular and Nanostructural Biophysics, BioNanoPark Ltd., Dubois 114/116, 93-465 Lodz, Poland

Corresponding author: marta.walczynska@p.lodz.pl, tel. no. +48426313064

Abstract

Nematode *Caenorhabditis elegans (C. elegans)* was used to investigate the impact of silver nanoparticles (SNP), multiwalled carbon nanotubes (MWCNT) and polyamidoamine dendrimers (PAMAM) used in concentration of 10^{10} particle/mL. Population based observations and gene expression analysis were employed in this study. SNP and PAMAM caused decrease in the number of live nematodes and their body length, but MWCNT did not affect the population of nematodes. Gene expression analysis revealed significant changes caused by the presence of all studied nanomaterials, and the results strongly suggest a specific metabolic response of the nematode organism to exposure to various nanomaterials. It was shown that *C. elegans* is a very sensitive organism capable to respond specifically to the

exposure to some nanomaterials and therefore could be considered as a possible biosensor for early warning of presence of some nanoparticles.

Key words:

Multiwalled carbon nanotubes, silver nanoparticles, dendrimers, toxicity, bioindicator, *C. elegans*

Acknowledgements

Nematode *C. elegans*, strain N2 and bacteria *E. coli*, strain OP50 were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

Funding

The authors received no financial support for the research, authorship and publication of this article

Declaration of Conflicting Interest

The Author(s) declare(s) that there is/are not conflict(s) of interest".

Introduction

Products of nanotechnology help overcome the shortcomings of traditional materials and therefore their production and use are the subject of intensive research and industrial activities. Unfortunately, with the intensive development of nanotechnology there is increasing threat to living organisms exposed to nanoparticles. Toxicology studies of engineered nanoparticles are well documented at least for simple homogenous silicone, metallic, metal oxide or carbon based nanoparticles (Bahadar et al. 2016), (Srivastava, Gusain, and Sharma 2015). Some reviews were made for selected nanoparticles, for example graphene (Ou et al. 2016) or dendrimers (Kaur et al. 2016). Considering the above, it is important to be aware of the necessity of monitoring of the presence of free-living nanoparticles in our

environment. A good example of the possibility of selective monitoring of the presence of nanoparticles is the report by J.H. Kim (Kim et al. 2017) showing the feasibility of implementing the *C. elegans* nematode as a biosensor selective for silver nanoparticles. The use of the model organism of nematode in nanoparticle toxicological studies is described in several papers, for example it was found, that graphene oxide, but not reduced graphene, can cause reproductive failure (Eom et al. 2015). Another set of reports describes the use of *C. elegans* for study of impact of other forms of carbon based nanomaterials (Chen, Hsiao, and Chou 2013) (Eom et al. 2015) (Chatterjee et al. 2014) (Miyako et al. 2015) (Cha, Lee, and Choi 2012) or silver (Luo et al. 2017) (Soria et al. 2015) and gold (Tsyusko et al. 2012) nanoparticles. Most of reports concern engineered nanoparticles especially prepared for the designed study, while in our environment usually are present various commercially available nanoparticles. The present study is devoted to evaluate the response of *C. elegans* to exposure to three commercially available nanparticles, i.e. multiwalled carbon nanotubes (MWCNT), silver nanoparticles (SNP) and poly(amidoamine) dendrimers (PAMAM). These samples represent groups of carbon, metal and polymer nanomaterials investigated for potential use of the nematode as a selective biosensor useful for monitoring the presence of nanoparticles. The nematode population was characterized by the number of live and dead animals and by the length of their bodies. Additionally an oligonucleotide microarray technique was used to evaluate changes in nematode gene expression.

Materials and Methods

Nanoparticles:

Multiwalled carbon nanotubes (MWCNT) (CAS Number 308068-56-6), Silver nanoparticles (SNP) as a powder with grain size below 100 nm, containing PVP as a dispersant (CAS Number 7440-22-4) and PAMAM dendrimers of fourth generation with ethylenediamine core

and surface amino groups (CAS Number 163442-67-9) were purchased from Sigma – Aldrich.

Biological materials:

Nematode *C. elegans,* strain N2, was used for the study. Bacteria *E. coli,* strain OP50 were used for breeding nematodes. Both, nematodes and bacteria were obtained from the Caenorhabditis Genetic Center, USA, program number P40 OD010440 ("Https://cbs.umn.edu/cgc/home." 2016).

Chemicals:

Magnesium sulphate, sodium hypochlorite and nickel sulphate were purchased from Chempur Poland. Agar, yeast extract and peptone were delivered by BTL Poland. Potassium chloride, sodium chloride, isopropanol, dipotassium phosphate, monopotassium phosphate, disodium phosphate, potassium permanganate and potassium sulphate were from POCH Poland. Guanidine hydrochloride, cholesterol, glucose, sodium hydroxide, propidium iodide and Triton X-100 were from Sigma-Aldrich Poland, and TRIzol was from Thermo Fisher Scientific USA. High quality water (Milli Q system) was used for buffers and other liquids preparation.

Characterization of nanoparticles:

Size of multiwalled carbon nanotubes and silver nanoparticles were determined with the use of Quanta 250 (FEI) scanning electron microscope. Size of PAMAM dendrimers was estimated with Zetasizer Nano-ZS90 (Malvern) instrument based on the phenomenon of dynamic light scattering and using the Stokes-Einstein formula (Frisken 2001) for size calculation. Elemental composition of nanoparticles was assessed with EDS (EDAX) spectrometer equipped with detector OCTANE PRO series and TEAM software. The spectrometer was an integral part of Quanta 250 system.

Culture of nematodes C. elegans:

Optical microscope GX 71 (Olympus) equipped with CCD camera and Stream Essentials software was used for observation of cultured nematodes *C. elegans*. All operations with the nematode culture were carried out in accordance with the procedures described in WormBook (Stiernagle 2006). The culture was run in 6-wells microplates (NUNC) containing Nematode Growth Medium (NGM- consisting of 1.7% agar, 0.25% peptone, 50 mM NaCl, 1 mM $CaCl_2$, 25 mM $KHPO_4$, 5 mg/l cholesterol, 1 mM $MgSO_4$).The NGM was supplemented with *E. coli* cells (suspension with OD=1 at 620 nm applied in 14 µl volume), and preincubated for 24 h at 20^{o}C before nematode seeding. Nematodes were then applied onto the NGM surface (about 40 animals per well), were cultivated for 48 hours and then used for synchronization of breeding. For experiments with nanoparticles, the NGM surface was supplemented with *E. coli* cells (as above) and with the required amount of nanoparticles of interest and then cultivated for 48 hours before application of synchronized animals in L1 form.

Synchronization of nematode culture started from extraction of eggs from adult animals by treatment with sodium hypochlorite in combination with sodium hydroxide (Porta-de-la-Riva et al. 2012). The collected eggs were then suspended in M9 buffer (0.3% KH_2PO_4, 0.6% Na_2HPO_4, 0.5% NaCl, 1mM $MgSO_4$) and incubated overnight at 20^{o}C with fairly vigorous shaking to obtain starved L1 animals. After that worms were pelleted and supernatant, containing dauer pheromone accumulated during starvation, was removed, and the animals were applied in equal volumes onto NGM surfaces containing nanoparticles.

Population-based studies:

An effect of the presence of nanoparticles on the nematode *C. elegans* population was examined for three kinds of nanoparticles: MWCNT, SNP and PAMAM, each used in concentrations of 10^{10} particles per mL. Every experiment was accompanied by two controls. The first one was carried out under standard culture conditions (Control) in the absence of any additional stress factor. The second, referred to as the positive control (Control+), was carried

out in the presence of nickel sulphate used at a concentration of 1 mM. At this concentration nickel sulphate does not cause bacteria mortality higher than 30%. In separate experiments the effect of nanoparticles on the survival of bacteria was also examined and for the used concentrations we found them less toxic than nickel sulphate. The nematodes were exposed to nanoparticles up to 72 hours, but 24 and 48 hours' time points were also used for the inspection of animal's population. The inspection was done by recording short movies (1 min.) with the use of GX 71 microscope and HyperCam2, free software program ("Https://hypercam.en.softonic.com" 2012). The movie was then edited with VirtualDub ("Http://www.virtualdub.org" 2011), free software program, to obtain separate frames and analyse the pictures in respect to the number of alive and dead animals and the size of live animals, with the use of Image J software ("Https://imagej.nih.gov/ij/" 2012). Additionally staining of dead nematodes with propidium iodide was employed (Ferreira et al. 2015). For this purpose after 72 hours of the culture propidium iodide (20 μM) was applied and after 15 minutes of incubation in the dark dead nematodes were observed with fluorescence microscope GX 71.

RNA isolation and characterization:

To analyze the nematodes *C. elegans* transcriptome, the animals were collected from the surface of the NGM after 72 hours of the culture. Nematodes were collected using M9 buffer/0.1% Triton-X, and then were washed three times with M9 buffer and after centrifugation were subjected to lysis with TRIzol (commercially available mixture of phenol and guanidine isothiocyanate and other compounds that lead to lysis of the cells and inactivate endogenous RNase)(Chomczynski and Sacchi 1987) .

In the next step chloroform was added to extract proteins and to separate DNA from RNA. Centrifugation in the presence of chloroform resulted in the formation of 3 layers, and thus the separation of the lysate components (RNA, DNA, proteins). The upper aqueous fraction

was RNA. This fraction, after harvesting, was treated with isopropanol to precipitate the RNA and its concentration. The final step of RNA isolation was drying the precipitate to remove chloroform and then dissolving in RNAse free water. The isolated RNA was then analyzed in respect to its purity and integrity with 2100 type Bioanalyzer (Agilent) according to the manufacturer procedure with the use of Agilent RNA 6000 Nano Kit. Only high quality RNA samples were used for transcriptome analysis.

Transcriptome analysis:

Changes in gene expression of the whole body of nematode *C. elegans* were analysed using oligonucleotide microarrays for double colour labelling of RNA probes (*C. elegans* (V2) Gene Expression Microarray, 4x44 K - Agilent). The array comprises four identical sections containing 43,803 oligonucleotides representing the whole genome in duplicate and additionally selected oligonucleotides used as markers for control of the hybridization process. The preparation of RNA molecular probes, hybridization procedure and subsequent washing of microarrays was performed using dedicated chemicals according to the manual supplied by Agilent. The hybridized microarrays were visualized for analysis with SureScan Agilent scanner and analysed with GeneSpring 12.5 software, both products of Agilent.

Genes with altered expression level and corresponding metabolic pathways were identified as a result of the analysis. The results are presented as the fold change of expression of the particular gene in respect to the expression of this gene in control animals.

Statistical analysis:

All experiments on nematode population were repeated at least six times and the results are presented as the mean ± standard deviation of mean followed by statistical analysis performed with ANOVA test with Bonferroni correction for multiple comparisons. The difference between mean values was considered as statistically relevant with significance level of $p<0.05$.

Results

Quality of commercially available nanoparticles:

The size of silver nanoparticles (SNP) and diameter of multiwalled carbon nanotubes (MWCNT) were estimated with the use of scanning electron microscopy (FEI Quanta 250), whereas the size of PAMAM dendrimers was determined by dynamic light scattering (Zetasizer Nano-ZS90 (Malvern)).

The SEM images for MWCNT and SNP are presented on Figure 1 a and b respectively:

a ….. b

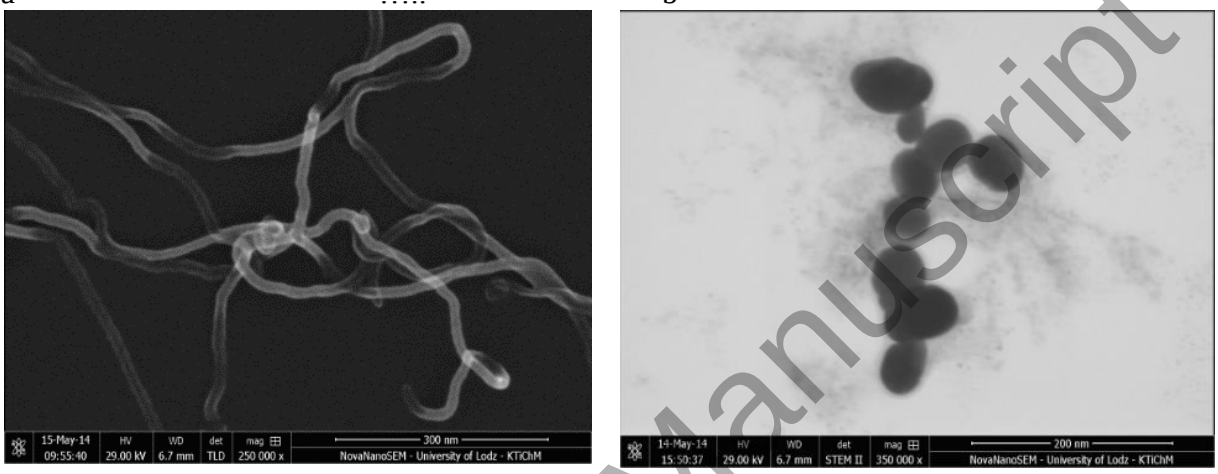

Figure 1 The SEM images for: A- MWCNT and B - SNP

The obtained results are gathered in the Table 1 in comparison with the data provided by the supplier.

Elemental composition of the nanoparticles was assessed by X-ray spectrometry with EDS spectrometer (see Table 2). Having regard to the contribution of the material of SEM mount specimen holder to the kind and amount of detected elements, results of our analysis were in good agreement with the chemical composition and purity declared by the supplier.

Population-based studies:

Three parameters were employed for evaluation of the nematode population. These are: increments of the nematodes number and the number of dead animals, as well as the length of the body of living animals. The nematode population was inspected immediately after start of the synchronized breeding and after 24, 48 and 72 hours. The positive control in the presence

of 1 mM nickel sulphate reduced the animals number and size very significantly, and induced very significant increase in the number of dead animals. This results were not considered within the below presented statistical analysis.

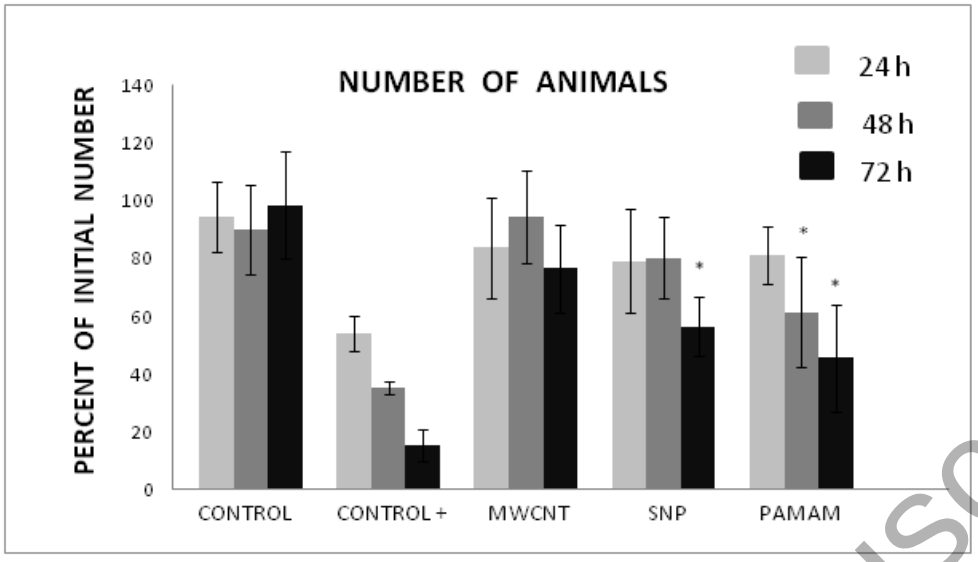

Figure 2 Analysis of the number of animals in populations

The first parameter characterizing population of nematodes was expressed as the number of alive animals at each time point in respect to the time 0 (Figure 2). The dead animals were also identified by staining with propidium iodide (Figure 3).

a b

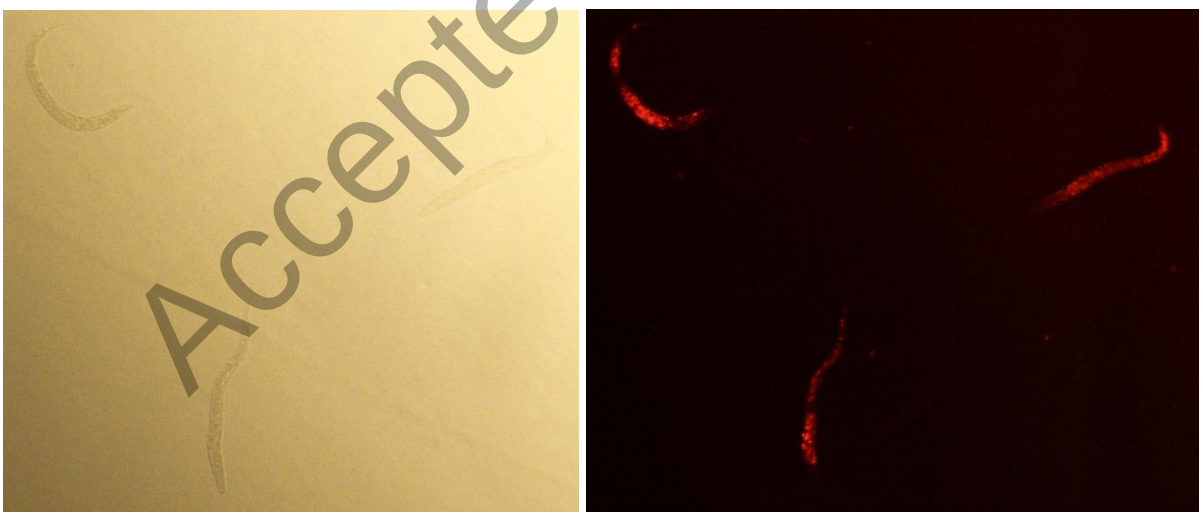

Figure 3 Animals seen in A - the bright field of view and B - propidium iodide stained dead animals seen with fluorescence microscope

The statistical analysis of obtained results implemented with one-way analysis of variance ANOVA showed that the differences between mean values were statistically significant with the level of significance $p < 0.0106$ and $p<0.0001$ for 48 and 72 hours, respectively. Using Bonferroni correction for multiple comparisons we found, that the difference was statistically relevant for comparison of control and SNP after 72h ($p<0.01$), and control and PAMAM after 48h ($p<0.05$) and 72h ($p<0.01$). MWCNT nanoparticles did not significantly affect the animals number.

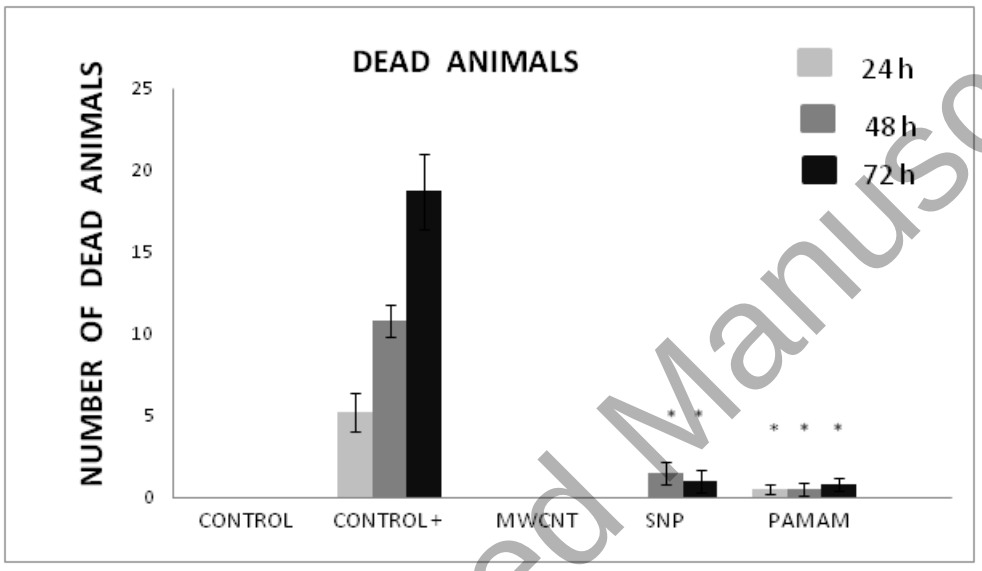

Figure 4 Analysis of the number of dead animals in populations

The next parameter described the number of dead nematodes during the time course of experiment. We did not find any dead animals in control and MWCNT treated populations, but in contrast to that SNP and PAMAM treated population presented singular dead animals. In this cases it was between 2 and 5 percent of initial number of animals, whereas for positive control animals, treated with nickel sulphate, it was between 10 and 50 percent.

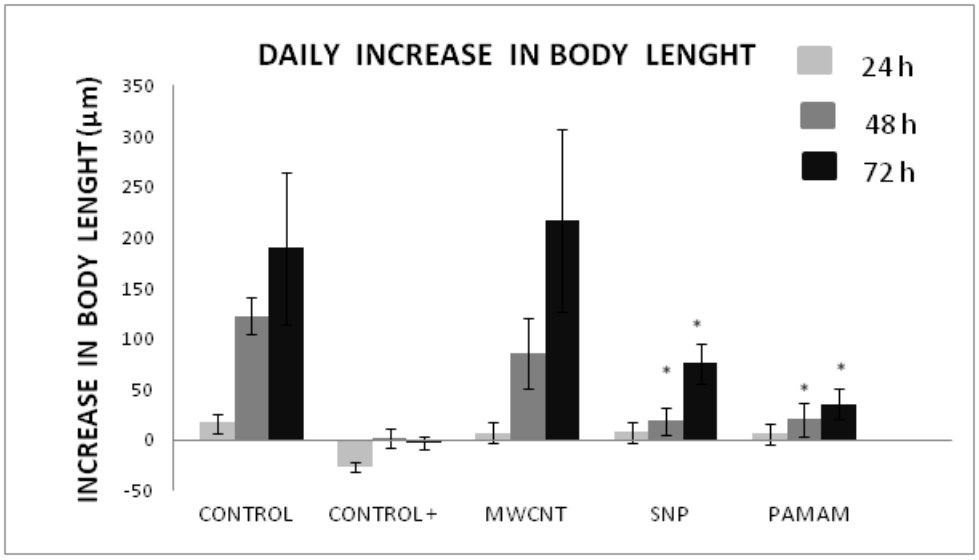

Figure 5 Daily increase in the body length in populations

The third parameter characterizing the nematodes populations was animal's body length expressed in micrometers. The Figure 5 presents the daily increase in the body length observed in the populations. When nickel sulphate was used (positive control) no increase in the body size was observed. All other cases showed an increments in the body length in the subsequent days, but the presence of MWCNT did not affect this process when compared with control population, while treatment with SNP and PAMAM resulted in very relevant reduction in this process. The statistical analysis showed no difference after 24 hours and very significant differences between control and SNP and PAMAM treated populations after 48 and 72 hours ($p<0.0001$ for both). Using Bonferroni correction for multiple comparisons we found, that the difference was statistically relevant for comparison of control and SNP treated animals after 48h and 72h ($p<0.01$ and $p<0.05$, respectively), and control and PAMAM treated animals after 48h ($p<0.01$) and 72h ($p<0.01$). MWCNT nanoparticles did not affect significantly the body length increase.

Transcriptome analysis:

The transcriptome analysis showed that all investigated nanoparticles induced changes in expression of genes of nematodes (see table 3). In the case of MWCNT we found changes in

expression of 16 genes (9 up regulated and 7 down regulated) representing 7 metabolic pathways. It makes about 7 percent of active genes in these pathways. In the case of SNP we observed changes in expression of 43 genes (34 over expressed and 9 suppressed) of 9 pathways, and it represents about 12 percent of the active genes of these pathways. Finally in the case of PAMAM changes in expression of 47 genes (29 up regulated and 18 suppressed) of 6 pathways were recorded, and it is about 27 percent of active genes of these pathways. It is worth to note, that no one pathway with altered gene expression was common for exposure to the investigated nanoparticles, although some genes were common for pairs of nanoparticles (see Table 4):

Discussion

In the present study the multicellular organism *C. elegans* was used to observe a toxic effect of commercially available nanoparticles i.e. multiwalled carbon nanotubes (MWCNT), silver nanoparticles (SNP) and poly(amidoamine) dendrimers (PAMAM). The nanoparticles have been tested for their size and chemical composition and the results were compared with those provided by the manufacturer or distributor. It was found that MWCNT were slightly larger (medians: 10.4 nm vs 6.6 nm declared by the manufacturer). In the case of SNPs our results also show that silver nanoparticles are slightly larger than those declared by the supplier, while the PAMAM dendrimers fit well with the declared size. Having regard to the contribution of the material of SEM mount specimen holder to the kind and amount of detected elements, results of our analysis were in good agreement with the chemical composition and purity declared by the supplier.

Taking into account the size, structure and density of the investigated nanoparticles, we calculated their number in suspension per volume unit, and the nematodes were exposed to nanoparticles present in the constant concentration of 10^{10} particles/mL.

The use of SNP and PAMAM nanoparticles in such a low concentration, however, resulted in statistically significant differences in the behaviour of the nematode population compared to the control culture. For PAMAM dendrimers both the number of live nematodes and the increase of their body length in consecutive days were lower than in control after the second day of culture. For SNP the number of live nematodes was lowered after the third day, although the body length was lowered after the second day. This changes were well correlated with the observed number of dead animals. Similar results have been recently reported for silver nanoparticles (Luo et al. 2017). The surprising result is the lack of an analogous effect of MWCNT on nematode population, but it is in good agreement with recently published data (Eom et al. 2015) where MWCNT used in concentration below 500 mg/L did not affect mortality of wide type *C. elegans*. The positive control, presence of nickel sulphate at 1mM concentration, always inhibited the development of the nematode population.

In addition to observation of the development of the nematode population, changes in expression of genes induced by exposure to the nanomaterials were analysed. The total transcriptome of whole animals after 72 hours of exposure to MWCNT, SNP or PAMAM was analysed in respect to control animals, and it was found that all nanoparticles caused changes in gene expression. Despite the fact, that carbon nanotubes have a minor or no effect on nematode's population, 16 genes of 7 metabolic pathways were affected. It makes about 7% of all active genes belonging to these metabolic pathways. In contrast, silver nanoparticles triggered changes in 43 genes of 9 pathways while PAMAM dendrimers were responsible for changes in expression of 47 genes belonging to 6 pathways. It makes about 12% and 27% of all active genes in respective pathways for SNP and PAMAM respectively. It is worth noting that none of the pathway with altered genes was common for expression to all three kinds of nanoparticles, but some genes were similarly altered by pairs of nanoparticles (see table 4). These initial observations of the transcriptome strongly suggest a specific metabolic response

of the nematode organism to exposures to various nanoparticles, although at this stage of the study we cannot unambiguously assess the weight of changes in the level of gene expression and their effect on the development of the nematode population. It seems that the obtained results confirm our earlier observations regarding the specific cell response to contact with nanoparticles (Walkowiak, Komorowski, and Walkowiak-Przybyło 2012). Unfortunately, it is not possible to compare the results of changes in gene expression caused by nanoparticle interaction with previously published results since the only currently available report (Eom et al. 2015) concerns only MWCNT but used at several times higher concentration (1 mg/L vs. 0.025 mg/L). Considering, however, the similarity between changes in nematode's population caused by the exposure to SNP and PAMAM and the fact that expressions of the same genes in the glycolysis pathway are altered in both cases, it can be argued that this pathway has a significant impact on the correct functioning of the organism.

Conclusions

Summing up, nematode *C. elegans* is a very sensitive organism capable of indicating the presence of some of nanoparticles even in a very low concentration (for example 10^{10} particles/mL for SNP and PAMAM) and could thus be used as a biosensor for early warning about the presence of these nanoparticles. Unfortunately, as our research shows, the universality of such a biosensor is limited, because not all nanoparticles have similarly strong influence on the nematode population. On the other hand, this model organism can serve as a convenient tool for studying the effects of nanoparticles on the functioning of the multicellular organism, and specificity of metabolic response to exposure to nanoparticles is an interesting finding with potential use to in identification of nanoparticles (Kim et al. 2017).

References

Bahadar, Haji, Faheem Maqbool, Kamal Niaz, and Mohammad Abdollahi. 2016. "Toxicity of Nanoparticles and an Overview of Current Experimental Models." *Iranian Biomedical Journal*. doi:10.7508/ibj.2016.01.001.

Cha, Yun Jeong, Jaesang Lee, and Shin Sik Choi. 2012. "Apoptosis-Mediated in Vivo Toxicity of Hydroxylated Fullerene Nanoparticles in Soil Nematode Caenorhabditis Elegans." *Chemosphere* 87 (1). Elsevier Ltd: 49–54. doi:10.1016/j.chemosphere.2011.11.054.

Chatterjee, Nivedita, JiSu Yang, Hyun-Mi Kim, Eunhye Jo, Phil-Je Kim, Kyunghee Choi, and Jinhee Choi. 2014. "Potential Toxicity of Differential Functionalized Multiwalled Carbon Nanotubes (MWCNT) in Human Cell Line (BEAS2B) and Caenorhabditis Elegans." *Journal of Toxicology and Environmental Health. Part A* 77 (22–24): 1399–1408. doi:10.1080/15287394.2014.951756.

Chen, Po Hsuan, Kuang Ming Hsiao, and Cheng Chung Chou. 2013. "Molecular Characterization of Toxicity Mechanism of Single-Walled Carbon Nanotubes." *Biomaterials* 34 (22). Elsevier Ltd: 5661–69. doi:10.1016/j.biomaterials.2013.03.093.

Chomczynski, Piotr, and Nicoletta Sacchi. 1987. "Single-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction." *Analytical Biochemistry* 162 (1): 156–59. doi:10.1016/0003-2697(87)90021-2.

Eom, Hyun Jeong, Carlos P. Roca, Ji Yeon Roh, Nivedita Chatterjee, Jae Seong Jeong, Ilseob Shim, Hyun Mi Kim, et al. 2015. "A Systems Toxicology Approach on the Mechanism of Uptake and Toxicity of MWCNT in Caenorhabditis Elegans." *Chemico-Biological Interactions* 239. Elsevier Ireland Ltd: 153–63. doi:10.1016/j.cbi.2015.06.031.

Ferreira SR, Mendes TAO, Bueno LL, de Araújo JV, Bartholomeu CD, Fujiwara RT. 2015. A "New Methodology for Evaluation of Nematode Viability" *Hindawi Publishing Corporation, BioMed Research International*, Volume 2015, Article ID 879263, 7 pages http://dx.doi.org/10.1155/2015/879263

Frisken, Barbara J. 2001. "Revisiting the Method of Cumulants for the Analysis of Dynamic Light-Scattering Data." *Applied Optics*. doi:10.1364/AO.40.004087.

"Http://www.dendritech.com/." 2015.

"http://www.sigmaaldrich.com/catalog/product/aldrich/576832?lang=pl&region=PL." 2015.

"Http://www.virtualdub.org." 2011.

"Https://cbs.umn.edu/cgc/home." 2016.

"Https://hypercam.en.softonic.com." 2012.

"Https://imagej.nih.gov/ij/." 2012.

"Https://www.chasmtek.com/." 2015.

Kaur, Daljeet, Keerti Jain, Neelesh Kumar Mehra, Prashant Kesharwani, and Narendra K. Jain. 2016. "A Review on Comparative Study of PPI and PAMAM Dendrimers." *Journal of Nanoparticle Research* 18 (6). Springer Netherlands: 1–14. doi:10.1007/s11051-016-3423-0.

Kim, J H, S H Lee, Y J Cha, S J Hong, S K Chung, T H Park, and S S Choi. 2017. "C. Elegans-on-a-Chip for in Situ and in Vivo Ag Nanoparticles' Uptake and Toxicity Assay." *Sci Rep* 7: 40225. doi:10.1038/srep40225.

Luo, Xun, Shengmin Xu, Yaning Yang, Yajun Zhang, Shunchang Wang, Shaopeng Chen, An Xu, and Lijun Wu. 2017. "A Novel Method for Assessing the Toxicity of Silver Nanoparticles in Caenorhabditis Elegans." *Chemosphere* 168: 648–57. doi:10.1016/j.chemosphere.2016.11.011.

Miyako, Eijiro, Svetlana A. Chechetka, Motomichi Doi, Eiji Yuba, and Kenji Kono. 2015. "In Vivo Remote Control of Reactions in Caenorhabditis Elegans by Using Supramolecular

Nanohybrids of Carbon Nanotubes and Liposomes." *Angewandte Chemie - International Edition* 54 (34): 9903–6. doi:10.1002/anie.201504987.

Ou, Lingling, Bin Song, Huimin Liang, Jia Liu, Xiaoli Feng, Bin Deng, Ting Sun, and Longquan Shao. 2016. "Toxicity of Graphene-Family Nanoparticles: A General Review of the Origins and Mechanisms." *Particle and Fibre Toxicology*. doi:10.1186/s12989-016-0168-y.

Porta-de-la-Riva, Montserrat, Laura Fontrodona, Alberto Villanueva, and Julián Cerón. 2012. "Basic Caenorhabditis Elegans Methods: Synchronization and Observation." *Journal of Visualized Experiments : JoVE*, no. 64: e4019. doi:10.3791/4019.

Soria, Cristina, Teresa Coccini, Uliana De Simone, Loredana Marchese, Irene Zorzoli, Sofia Giorgetti, Sara Raimondi, et al. 2015. "Enhanced Toxicity of Silver Nanoparticles in Transgenic Caenorhabditis Elegans Expressing Amyloidogenic Proteins." *Amyloid : The International Journal of Experimental and Clinical Investigation : The Official Journal of the International Society of Amyloidosis* 6129 (October): 1–8. doi:10.3109/13506129.2015.1077216.

Srivastava, Varsha, Deepak Gusain, and Yogesh Chandra Sharma. 2015. "Critical Review on the Toxicity of Some Widely Used Engineered Nanoparticles." *Industrial and Engineering Chemistry Research*. doi:10.1021/acs.iecr.5b01610.

Stiernagle, Theresa. 2006. "Maintenance of C. Elegans." *WormBook*, no. 1999: 1–11. doi:10.1895/wormbook.1.101.1.

Tsyusko, Olga V., Jason M. Unrine, David Spurgeon, Eric Blalock, Daniel Starnes, Michael Tseng, Greg Joice, and Paul M. Bertsch. 2012. "Toxicogenomic Responses of the Model Organism Caenorhabditis Elegans to Gold Nanoparticles." *Environmental Science and Technology* 46 (7): 4115–24. doi:10.1021/es2033108.

Walkowiak, Bogdan, Piotr Komorowski, and Magdalena Walkowiak-Przybyło. 2012. Means of identification of materials engineering products or nanotechnologies. 223224, issued 2012.

Table 1. Obtained mean values of diameter of MWCNT, SNP and PAMAM with the data given by the suppliers.

	MWCNT		SNP		PAMAM	
	Manufacturer's (SouthWest Nanotechnologies Inc. data ("Https://www.chasmtek.com/" 2015)	**SEM measurements data**	**Supplier (Sigma) data** ("http://www.sigmaaldrich.com/catalog/product/aldrich/576832?lang=pl&region=PL" 2015)	**SEM measurements data**	**Manufacturer's (Dendritech Inc.) data** ("Http://www.dendritech.com/" 2015)	**DLS measurements data**
Range [nm]	(diameter of 75% of MWCNT < 9 nm; diameter of 90% MWCNT < 12.2 nm	1.9 ÷ 39	diameter of 99% of SNP< 100 nm	22.8 ÷ 123.3	Lack of data	4 ÷ 6
mean ±standard deviation [nm]	Lack of data	12.3 ± 7.7	Lack of data	59.9 ± 19.4	4.5	5 ± 1
Median [nm]	6.6	10.4	Lack of data	55.3	Lack of data	4.9

Table 2. Elemental composition of the nanoparticles assessed with EDS spectrometer (EDAX OCTANE PRO).

MWCNT			SNP			PAMAM		
Element	**Mass concentration [%]**	**Error [%]**	**Element**	**Mass concentration [%]**	**Error [%]**	**Element**	**Mass concentration [%]**	**Error [%]**
C	58.98	9.6	**Ag**	100	1.25	**C**	36.03	6.18
Al	38.77	2.32				**N**	28.38	13.14
Cu	2.25	14.43				**O**	35.59	11.82

Table 3. List of genes with altered expression and corresponding metabolic pathways. The value of fold change is given in brackets, where negative value corresponds to gene suppression.

Metabolic pathway	No. of active genes	MWCNT	SNP	PAMAM
		genes with changed expression (fold change)	genes with changed expression (fold change)	genes with changed expression (fold change)
Aging	3	*daf-2 (3.18)* *daf-16 (1.01)* *smk-1 (-2.13)*	-	-
Cytoplasmic ribosomal Proteins	63	*bet-1 (2.48)* *rpl-31 (-6.50)* *rpl-33 (1.01)* *ubq-2 (-2.08)* *rps-14 (1.01)* *rps-20 (1.01)*	-	*rpl-6 (3.11)* *rpl-10 (3.11)* *rpl-13 (-4.93)* *rpl-15 (9.31)* *rpl-17 (7.31)* *rpl-18 (-12.67)* *rpl-26 (2.87)* *rpl-32 (-2.74)* *rpl-43 (-7.90)* *rps-4 (2.25)* *rps-5 (5.42)* *rps-9 (-3.38)* *rps-12 (2.12)* *rps-13 (-5.57)* *rps-14 (1.04)* *rps-17 (2.06)* *rps-30 (-2.98)*
Insulin signaling pathway	8	*daf-2 (3.18)* *daf-16 (1.01)*	*age-1 (1.03)* *daf-16 (1.03)*	-
Dauer formation	23	*daf-2 (3.18)* *daf-12 (-5.15)* *daf-16 (1.01)*	-	-
Immune responses in the intestine	25	*daf-2 (3.18)* *daf-16 (1.01)* *spp-1 (-2.44)*	*daf-16 (1.03)* *est-4 (-1.08)* *atf-7 (-7.08)* *lys-7 (1.03)*	-
Meta pathway signal transduction	107	*par-1 (1.01)* *lin-45 (1.01)* *daf-2 (3.18)* *daf-12 (-5.15)* *daf-16 (1.01)* *lag-1 (1.01)*	*cwn-1 (3.25)* *cwn-2 (2.13)* *dsh-2 (71.43)* *par-1 (-63,35)* *let-23 (-2,14)* *glp-1 (1.03)* *dlk-1 (1.03)* *daf-16 (1.03)* *age-1 (1.03)*	-
Mitochondrial unfolded-Protein response	18	*hsp-60 (-2.17)* *dnj-10 (2.12)*	-	-
II division – first embryonic mitosis	124	-	*plk-1 (2.01)* *arp-1 (1.03)* *atad-3 (-2.06)* *mel-28 (1.03)*	-

			rbx-1 (5.96) *dnc-1 (1,13)* *kle-1 (1.03)* *knl-3 (-1.06)* *ndc-80 (1.03)* *rod-1 (1.03)* *mfd-1 (-1.10)* *unc-59 (1.03)* *mlc-4 (1.03)* *him-1 (1.03)*	
Fatty acid biosynthesis	17	-	*pyc-1 (2.01)* *acly-1 (1.03)* *ech-6 (1.03)* *unc-11 (1.03)*	-
Fatty acid Beta Oxidation	21	-	*tpi-1 (7.04)* *ech-1.2 (1.03)* *ech-6 (1.03)* *atol-1 (1.03)*	-
Vulval development	18	-	*sur-2 (1.03)* *sur-6 (1.01)* *let-23 (-2.40)*	-
Common Glycogen Metabolism	21	-	*let-92 (-6.01)* *paa-1 (2.16)* *sur-6 (1.01)*	-
Glycolysis	25	-	*fbp-1 (1.03)* *tpi-1 (7.40)* *pyc-1 (1.03)*	*hxk-1 (3.07)* *acl-4 (-6.67)* *fbp-1 (-3.67)* *aldo-2 (4.10)* *pyc-1 (3.07)* *pck-1 (2.27)*
DNA replication	25	-	-	*orc-1 (-4.99)* *mcm-3 (3.17)* *mcm-2 (-18.54)* *arpa (2.06)* *rfc-1 (-1.04)* *f10c2.4 (2.69)* *cdc-6 (2.04)*
LIN-12-Notch Lateral signaling	15	-	-	*let-23 (2.04)* *lst-2 (2.89)* *dpy-23 (2.19)* *mpk-1 (2.79)* *unc-101 (2.02)*
Sex determination	17	-	-	*sex-1 (-2.89)* *sea-1 (7.70)* *sdc-3 (1.04)* *fem-1 (3.00)* *sel-10 (2.03)*
Translation factors	31	-	-	*eif-3.H (-2.08)* *eif-3.G (-5.72)* *ife-3 (2.67)* *eef-1g (-2.13)*

				eft-2 (-7.23) *eif-2α (-2.03)* *eif-3b (2.20)*
Total number of genes (+ upregulated, - downregulated)		16 (+9, -7)	43 (+34, -9)	47 (+29, -18)

Table 4. Genes with altered expression and corresponding metabolic pathways common for pairs of MWCNT, SNP and PAMAM. The value of fold change is given in brackets, where negative value corresponds to gene suppression.

Metabolic pathway	MWCNT	SNP	PAMAM
Immune responses in the intestine	*daf-16 (1.01)*	*daf-16 (1.03)*	-
Meta pathway signal transduction	*par-1 (1.01)*	*par-1(-63.35)*	-
Insulin signaling pathway	*daf-16 (1.01)*	*daf-16 (1.03)*	-
Cytoplasmic ribosomal proteins	*rps-14 (1.01)*	-	*rps-14 (1.04)*
Glycolysis	-	*fbp-1 (1.03)* *pyc-1 (1.03)*	*fbp-1 (-3.61)* *pyc-1 (3.07)*