INTERACTION OF NANOPARTICLES WITH NEURAL STEM-AND TISSUE-TYPE CELLS

PhD thesis

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Budapest, 2015

“Anyone who doesn’t take truth seriously in small matters cannot be trusted in large ones either.”

- Albert Einstein

The main objective of the studies was to explore the reactions of different neural tissue cells to defined types of nanoparticles. The study focused on

- *the roles of chemical surface composition of otherwise identical nanoparticles*. To avoid variations by size and dissolution of biologically active compounds, particles with uniform size and non-toxic, (polystyrene, silica) core material but with different surface groups were probed in vitro on neural stem cells, stem cell-derived and primary neurons, astrocytes, microglia and brain microvessel endothelial cells.
- *the barrier function of the placenta against the invasion of differently functionalized NPs*. The distribution of negatively charged and PEG-passivated PS NPs in the placenta and embryonic brain was investigated 5 minutes and 4 days after a single intravenous injection of particles.
- *the roles of aging of nanoparticles in biological interactions*. The cellular uptake and viability effects of fresh and aged (shelf-life > 6 months) NPs were compared and were related to the physico-chemical changes of NPs during ageing.
- *the roles of shape of Ag NPs in neurotoxicity*. Ag NPs with different (sperical, cubic triangle, rod) shapes were synthezised, characterized and probed on neural stem cells.

Project summary

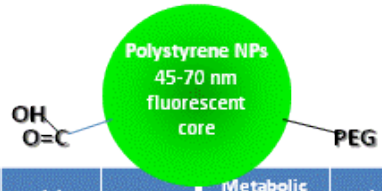
As nanotoxicology is in its infancy, basic data on safety of nanoparticles are missing even for those which are produced and used in large quantities. There are certain concerns regarding the environmental and human health risks of engineered nanomaterials (ENMs) in both unintended exposures, and medical applications. There is an urgent need for information on the mechanisms of nanoparticle interactions with physiological barriers and cells. Several studies showed that the nano materials could enter into the brain (Medina et al., 2007). Therefore, our main research focuses on the penetration of particles into the adult and developing central nervous system, and on the *in vitro* effects of nanoparticles (NPs) on different neural cell types including neurons, astrocytes, microglia, brain microvessel endothelial cells and neural stem cells. Among various physico-chemical properties known to influence the biological effects of nanoparticles, these studies focused on the role of chemical composition of NP surfaces. For this end, particles of the same (45-70 nm) size-range and of non-toxic core-material (silica or polystyrene) were included in the studies. The particles were thoroughly analysed before by using several methods including dinamic light scattering (DLS), Zeta-potential determination, differential centrifugal sedimentation (DCS), nanoparticle tracking analysis (NTA) and transmission electronmicroscopy (TEM).

Interaction of Nanoparticles with neural stem-and tissue-type cells

The interaction of 50 nm fluorescent core/shell silica NPs with neural tissue cells depended strongly on both, the surface charge of particles and the type of the interacting cells. “Passivating” the particle surfaces with polyvinyl-pyrrolidone (PVP) reduced the interactions with biological material resulting in reduced protein adsorption by NP surfaces, decreased toxicity and cellular uptake. The cellular effects of 50-70 nm polystyrene (PS) NPs with negatively charged (carboxylated) or PEG-passivated surfaces also showed important surface-dependent differences and cell-type dependent variations. Silica and PS NPs used within 6 months of synthesis proved to be not toxic to neural tissue cells up to extremely high doses. Uptake experiments using confocal spectrum analysis microscopy showed that neurons did not take up any particles, while microglial cells internalized a large amount of negatively charged particles but almost no particles with passivated (PEGylated or PVP coated) surfaces.

The *in vivo* tissue penetration of PS NPs was investigated in parallel studies by injecting 33μg/kg body weight NPs into the tail vein of pregnant mice, and analyzing tissue sections by confocal spectrum analysis microscopy. Significant differences were found in the short-term tissue invasion between carboxylated and PEG-coated PS particles. The distribution of PS-NPs in the adult mouse body is presented in PhD thesis of Kata Kenesei (PhD school of Molecular

medicine, Semmelweis University, Budapest). My work demonstrated that the placenta has a proper barrier capacity to protect the developing embryo from the entry of 45-70nm PS NPs. Regardless of functionalization, particles were not found in embryonic tissues, and were completely cleared from the placenta in a 4-day after injection period.



Cell type	Metabolic effect	toxicity	uptake	Metabolic effect	toxicity	uptake
NE-4C neural stem cells	-	-	-	-	-	-
NE-4C neurons	-	-	-	-	-	-
Primary neurons	-	-	-	-	-	-
Astrocytes	-	-	-	-	-	-
Brain endothel	↑	-	?	-	-	?
Microglia	↑	↑	↑ ↑ ↑	-	-	↑

Ageing: Particle aggregation; endotoxin adsorption

Cell type	Metabolic effect	toxicity	uptake	Metabolic effect	toxicity	uptake
NE-4C neural stem cells	↑	↑	↑	↑	↑	↑
Microglia	↑	↑↑	↑ ↑ ↑	↑	↑↑	↑ ↑ ↑

PEGylation does not prevent endotoxin adsorption by PS NPs

When experiments were repeated with “aged” NPs with shelf-life longer than 6 months, enhanced toxicity and cellular NP uptake were detected. “Aged” PS nanoparticles were taken up in large amounts by

microglia cells, regardless of the original surface composition, and were internalized also by neural stem cells. Endotoxin assays including *Limulus* ameobocyte clotting (LAL) tests and SDS-PAGE showed that both PEGylated and carboxylated PS NPs adsorbed significant amounts of bacterial lipopolysaccharide's (LPS). Nanoparticle size analyses proved the formation of large particle-aggregates during prolonged storage. Endotoxin accumulation and aggregation of particles were not prevented by PEG passivation of the nanosurfaces. The results call for introducing potent screening methods for detecting toxic contaminants of nanoparticles, especially those intended for nutritional and biomedical use.

Besides the non-toxic PS and silica NPs, the role of the shape of silver nanoparticles (Ag NPs) in interactions with living material was also investigated. Ag NPs with an average 35-50 nm size and with different geometries (spheres, cubes, triangles and rods) were prepared and characterized. The dissolution of Ag ions from particles was monitored by UV-Vis analysis and the cellular uptake of particles was investigated by transmission electron microscopy (TEM). Cell viability and TEM studies demonstrated the toxic effects of Ag NPs on neural stem cells with extreme toxicity for Ag nanorods. Coating Ag NPs with PVP slightly reduced toxicity of Ag spheres, but not that of Ag rods. The data demonstrated that the cellular toxicity of Ag NPs can be modified by adjusting the shape and chemical surface activation of the particles

The presented data may contribute to the development of safety guidelines for application of nanomaterials in rapidly developing medical nanotechnology.

The main findings of the work

- NPs with non-toxic (polystyrene or silica) core material and with a size of 45-70 nm, did not exert acute toxic effects on any of the investigated neural cells.
- The low-level toxicity (found at extremely high particle concentrations) was further decreased if the surface of particles were coated with non-ionic polymer molecules as poly-ethylene glycol (PEG) or polyvinylpirrolidon (PVP).
- The cellular responses to NPs reflected the physiological characteristics of different neural tissue cells:
  - neurons did not react to NP-loading with either metabolic or uptake responses
  - endothelial cells showed metabolic activation without cell damages
  - microglia cells displayed metabolic activation besides a significant uptake of NPs
- Passivation of NP surfaces with PEG or PVP resulted in a marked reduction of cell responses

- Aged PS NPs evoked different cell responses due to particle aggregation and accumulation of bacterial endotoxins on NP surfaces
- Passivation with PEG PS NP surfaces did not prevent endotoxin accumulation
- Silver NPs (35-50 nm) exerted shape-dependent toxic effects on neural cells with a toxicity-rank of spheres<cubes<triangles<rods. Toxicity was due to shape-dependent dissolution of Ag ions and the severe mechanical damages by rod-shaped NPs

Publications related to the PhD thesis

- Kumarasamy Murali, K. Kenesei, Yang Li, K. Demeter, Zs. Környei, E. Madarasz. Uptake and bio-reactivity of polystyrene nanoparticles is affected by surface modifications, ageing and LPS adsorption: in vitro studies on neural tissue cells, *Nanoscale*, 2015, 7, 4199 – 4210. Impact factor: 6.789.

- Izak-Nau E, Kenesei K, Murali K, Voetz M, Eiden S, Puntes VF, Duschl A, Madarász E. Interaction of differently functionalized fluorescent silica nanoparticles with neural stem- and tissue-type cells. *Nanotoxicology*. 2014, 8(1), 138-148. Impact factor: 7.336.

- Kata Kenesei, Kumarasamy Murali, Árpád Czéh, Jordi Piella, Victor Puntes, Emília Madarász. Effects of surface modifications on the distribution of polystyrene nanoparticles in mouse tissues, *Nanomedicine* (under review 2015).

Acknowledgements

There are many people I would like to acknowledge for their help and support. First and foremost, it gives me immense pleasure to thank my mentor Prof. Dr. Madarasz Emilia for giving me the opportunity to carry out my research project as a Marie Curie fellow and for the professional help in all stages of the work.

I would also like to thank Ms Kata Kenesei for helping me with microscopic studies and her suggestions and I could not forget to give huge thanks to all the past and present members of the Laboratory of Cellular and Developmental Neurobiology I have met along this "fantastic trip" for the great enthusiastic research and friendly atmosphere rendered. Especially I would like to thank Katalin Gaal, Lia Barabas and Piroska Nyamandi, for their excellent technical assistance.

I thank to my fellow 'NanoTOES members', especially Emilia Izak Nau and Yang li, for their research contributions.

My sincere thanks are also goes to Prof Albert Duschl (Coordinator, "EU FP7 Marie Curie Actions, and Network for Initial Training „NanoTOES" project) and Elisabeth Eppacher for their administrative support.

I would also give great thanks to my collaborative research labs at Bayer Technology Services GmbH (Leverkusen) at ICN (Barcelona), at Centre for BioNano Interactions (CBNI), University College Dublin,

Belfield, Dublin, Ireland and at NILU (Oslo) for the great research environment.

Dr Diana Boraschi (Laboratory of Innate Immunity and Cytokines, Institute of Protein Biochemistry, National Research Council, 80131 Naples, Italy) for the critical reading and correcting the manuscript of my first authorship paper.

I sincerely thank the EU FP7 Marie Curie Actions, Network for Initial Training NanoTOES (PITN-GA-2010-264506) for the financial assistance. (www.nanotoes.eu)

I wish to thank the Nikon Microscopy Center of Nikon Austria GmbH, and Auro-Science Consulting Ltd operating at the Institute of Experimental Medicine of Hungarian Academy of Sciences, (Budapest, Hungary),

Dr. Jorge MEJIA, PMR-LARN, Faculty of Sciences, Université de Namur ASBL, Belgium for CPS measurements.

Sincere thanks are also to Széles Csilla, Radó Nikolett, Benko gabriella, and Seregély Sára for their administrative support througout my project and to the staff from Secreatry office of Doctoral School of Semmelweis University, Hungary.

Last but not the least I am also very thankful to the experimental animals for their sacrifices without which the study would not have been possible, may god keep their soul in peace.

A warm thanks to all my friends in Hungary who have enriched my life abroad.

I am grateful for the love and tolerance of my dearest Mritti.
Last, but certainly not least, I would express a deep sense of gratitude especially to my dearest mom, who has sacrificed her life for my growth and success and to whom I owe my life for her constant love, encouragement, moral support and blessings. Although, she is miles away from me, but her love and support were always with me.
Finally, I would like to dedicate this thesis to my grandpa and to all my uncles, personally they have shown me unconditional love and support during my school days and they have played an important role in the development of my identity and shaping the individual that I am today.
I would like to finish with a quote that has helped me through the tough times over the last few years;

“Nothing is permanent in this wicked world, not even our troubles”.

-Charlie Chaplin.