Answers to Question Set 5

Interactions of nanostructures with biological matter

It is necessary to read and understand the papers. The lecture handouts are also helpful to understand what is important in the course. Note that the questions may require you to go beyond the papers specified here in order to find your answers.

Basics of nanotoxicology

A nanotoxicology review paper[1] may be useful. Here is a paper on the protein corona[2].

(1) In which way can we be exposed to nanoparticles? How can nanoparticles be eliminated from the body?

Entry routes – inhalation, ingestion, injection, skin permeation

Sources – combustion, skin creams (e.g. sun screen), BBQ, volcanoes

Elimination – primarily faeces and urine

(2) What are the basic factors determining the toxicity of nanoparticles?

- *Size, area, shape, chemistry, dissolution, crystal properties, agglomeration, surface energy / hydrophocity*

(3) What may happen to the nanoparticles when they enter a living organism? How may it affect the toxicity of the nanoparticles?

- *Proteins binding to the nanoparticle, protein corona; biased uptake of proteins*
- *Biodistribution – distribution to different organs, membrane structures etc.*
- *ROS formation causing DNA damage, membrane damage, neurotoxicity, ...*

(4) Currently in Europe, for new chemicals, the level of testing required is determined by the mass produced, with the lowest mass trigger currently set at 10kg per annum.

What is the volume of 10kg of GaP? How many nanowires (5 µm long, 50 nm in diameter) do 10 kg of GaP correspond to? How big would the surface area of the single GaP piece be (assuming a sphere)? How big would the total surface area of the nanowires be? (GaP density=4.138 g/cm^3)

- Volume of 10kg GaP is found by knowing that the density of GaP is 4138kg/m^3.

$$V_{sphere} = \frac{m}{\rho} = \frac{10kg}{4138kg/m^3} = 2.42 \cdot 10^{-3} m^3$$

- Comparing to the volume of one nanowire gives the number of nanowires corresponding to the volume of the sphere.

$$n = \frac{V_{sphere}}{V_{NW}} = \frac{\frac{4\pi}{3} R^3}{\pi r^2 L} = \frac{4}{3} \frac{R^3}{r^2 L}$$

We need the radius of the sphere.

$$R = \left(\frac{V_{sphere}}{\frac{4\pi}{3}}\right)^{1/3} = \left(\frac{2.42 \cdot 10^{-3} m^3}{\frac{4\pi}{3}}\right)^{1/3} = 83.3 \cdot 10^{-3} m$$

$$\Rightarrow n = \frac{4}{3}\frac{R^3}{r^2 L} = \frac{4}{3}\frac{(83.3 \cdot 10^{-3} m)^3}{(50 \cdot 10^{-9} m)^2 5 \cdot 10^{-6} m} = 61.7 \cdot 10^{15}$$

- Surface area of the sphere.

$$A_{sphere} = 4\pi R^2 = 4\pi(83.3 \cdot 10^{-3} m)^2 = 87.2 \cdot 10^{-3} m^2$$

- Total surface area of the nanowires is given by the number of nanowires times the area of each nanowire.

$$A_{NW} = 2\pi r L = 2\pi \cdot 50 \cdot 10^{-9} m \cdot 5 \cdot 10^{-6} m = 1.57 \cdot 10^{-12} m^2$$

$$A_{NW}^{TOTAL} = n 2\pi r L = 61.7 \cdot 10^{15} \cdot 1.57 \cdot 10^{-12} m^2 = 97 \cdot 10^3 m^2$$

- The ratio is now easily found.

$$ratio = \frac{A_{NW}^{TOTAL}}{A_{sphere}} = \frac{97 \cdot 10^3 m^2}{87.2 \cdot 10^{-3} m^2} = 10^6$$

Tools to study nanotoxicology

Here one paper on organ on a chip[3] and one paper on barcoded nanowires[4] are useful.

(1) What are the limitations and issues faced when using cell cultures or animal (or human) models for nanotoxicology studies?

- cell cultures: do not capture the whole process (uptake, biointeraction, elimination); does not give a true picture

- animal and human test subjects – ethical considerations

(2) In which way would organs on a chip be useful alternative to cell cultures or animal (or human) models for nanotoxicology studies? What limitations could be imagined for organs on a chip? How can the latter problem be addressed?

- realistic effects on the organ level; the effect of breathing motion on the uptake of nanoparticles

- no systematic effects; not exact replication of in-vivo conditions; different flow rates; different cell composition

- combine several organs on a chip to obtain a more comprehensive picture

(3) Why would barcoded nanoparticles be a useful tool in nanotoxicology? Explain the basic idea and give examples of uses.

- multiplexed experimental conditions; many different nanoparticles at the same time; nanoparticles added at different time points or through different administration routes (e.g. skin, ingestion, inhalation etc)

- biodistribution studies, i.e. where do the particles go as a function of size, chemistry etc

- with solid state fluorophores, better photo stability is the result

- nanowires with segments with different fluorescence

- nanoparticles with spectral barcodes, i.e. different mixture of colors

Usage of nanoparticles and nanostructures

Here several papers might be useful: nanoparticles for biomedicine[5, 6], nanowires for control of cell growth[7], nanowires as nanoinjection needles[8], phage display [9].

(1) In which ways can nanoparticles be useful for treatment?

- drug delivery (e.g. using antibodies attached to the nanoparticles for targeting of specific tissue or by using an external magnet to attract magnetic nanoparticles to a specific location)

- physical action to cells (heating, mechanical breakdown) inducing apoptosis (e.g. of cancerous cells)

(2) What is molecular imprinting?

- essentially artificial antibodies. Antigens are mixed into a resin which is subsequently crosslinked. The antigens are subsequently rinsed away. The remaining space now has specific affinity to the original antigens.

(3) In which ways can nanowires be useful for the development of neuroprostetics?

- guidance of neurons

- creation of surface for selective growth of cells

(4) Electroporation is a method to deliver molecules to cells. Why would hollow nanowires be better?

- electroporation relies on diffusion; an equilibrium is established between outside and inside; more controlled transport of reagents to the cytosol; it also opens up for extraction of a small sample from the cell for further analysis; electroporation may damage the cell

(5) What is phage display and how can it be used to create proteins with specific binding properties?

- Phage display is a method to display proteins on the surface of a virus. The gene for a protein is inserted in the viral genome so that it is expressed in the capsid.

The approach can be used for directed evolution in order to develop proteins with specific binding properties. A surface is exposed to viruses and subsequently rinsed. Those viruses that attach to the surface are collected and multiplied under mutating conditions. The procedure is repeated for these viruses. In this case, a slightly larger fraction of viruses binds to the surface. This is repeated until a sufficient number of viruses can be collected. With the viral DNA, the sequence can be identified for the protein that binds to the specified surface. The protein can therefore subsequently be

synthesized in its pure form using standard methods, *e.g.* recombinant DNA technologies.

DNA nanotechnology

Here these two papers might be useful [10] and [11].

(1) What is the basic mechanism that is used to create nanostructures using DNA?

- M13 viral DNA + crosslinking DNA oligomers. Use software to design structures. The software gives a list of oligos needed. Simply mix! The challenge is to image the structures.

(2) Give examples of potential uses of DNA nanostructures.

- drug delivery… boxes that contain active substances; the active substance can be released by changes in local chemistry (pH, salt, enzymes, etc) or by extermal stimulus (light).

(3) Compare chemical caging / caged compounds with DNA nanostructures.

- chemical caging consists of combining an active molecule with an inhibitor that can be removed using light. See ref [12] for an overview.

- using DNA one can build a real cage where molecules of interest can be stored; there is no chemistry done on the molecules that are put in the DNA box which may make things simpler; on the other hand, to control the exact number of molecules in each box may be a challenge. With a large box, the number of molecules would be Poisson distributed. Solutions to the problem that are worth considering may include benefitting from the confinement of the box to fit a specified number of molecules. Another solution may be to bind the molecules weakly inside the box to specific binding sites that are chemically defined in the wall of the box.

Concluding remarks

Effect of size ->

- area (dissolution speed increases with increased area/volume; depletion of proteins; surface reactivity)
- small particles can go where not larger particles can go (useful for drug delivery)
- bandgap changes with size; this has relevance for phototoxicity

Cells versus animals ->

Both animals and cells are needed to assess the (nano)toxicity of a given product.

Animals are complete living systems and the overall effect is tested. Not only the effect of the nanoparticles inside the animal, but also the pathway of the cells into the animal and information about where the nanoparticles go, and finally how they are cleared from the animal. However, the test is painful for the animal and the exact details the bio-nanointeraction can be difficult to follow.

Cells grown on a petri dish are not in their natural state. However, the detailed effects on the particular cells can be studied and there is no (or minimal depending on the origin of the cells) suffering of any lab animals.

Organ on a chip - Recent developments on organs and even groups of organs on a chip hold great promise. They combine a more realistic model system with the animal-ethically preferable in-vitro setup. Here cells are grown in a more realistic setting with several cells in a 3D structure combined with any motion and fluid flows that would be natural for the cells. For example a “lung on a chip” has shown that nanoparticles interact completely differently in a artificial lung tissue that is not moving and one that is moving [3].

References

1. Sharifi, S., et al., *Toxicity of nanomaterials*. Chemical Society Reviews, 2012. **41**(6): p. 2323-2343.
2. Cedervall, T., et al., *Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles*. Proceedings of the National Academy of Sciences of the United States of America, 2007. **104**(7): p. 2050-2055.
3. Huh, D., et al., *Reconstituting Organ-Level Lung Functions on a Chip*. Science, 2010. **328**(5986): p. 1662-1668.
4. Adolfsson, K., et al., *Fluorescent Nanowire Heterostructures as a Versatile Tool for Biology Applications*. Nano Letters, 2013. **13**(10): p. 4728-4732.
5. Zhang, E., et al., *Dynamic Magnetic Fields Remote-Control Apoptosis via Nanoparticle Rotation*. ACS Nano, 2014. **8**(4): p. 3192-3201.
6. Kempe, H. and M. Kempe, *The use of magnetite nanoparticles for implant-assisted magnetic drug targeting in thrombolytic therapy*. Biomaterials, 2010. **31**(36): p. 9499-9510.
7. Piret, G., M.-T. Perez, and C.N. Prinz, *Support of Neuronal Growth Over Glial Growth and Guidance of Optic Nerve Axons by Vertical Nanowire Arrays*. ACS Applied Materials & Interfaces, 2015. **7**(34): p. 18944-18948.
8. Xu, A.M., et al., *Quantification of nanowire penetration into living cells*. Nature Communication, 2014. **5**(3613): p. -.
9. Whaley, S.R., et al., *Selection of peptides with semiconductor binding specificity for directed nanocrystal assembly*. Nature, 2000. **405**(6787): p. 665-668.
10. Rothemund, P.W.K., *Folding DNA to create nanoscale shapes and patterns*. Nature, 2006. **440**(7082): p. 297-302.
11. Andersen, E.S., et al., *Self-assembly of a nanoscale DNA box with a controllable lid*. Nature, 2009. **459**: p. 73.
12. Ellis-Davies, G.C.R., *Caged compounds: photorelease technology for control of cellular chemistry and physiology*. Nature Methods, 2007. **4**: p. 619.