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Subject | Minutes of WG1/WG3 joint Workshop on

"Synthesis and functionalisation of magnetic nanoparticles for hyperthermia and radiotherapy"

of COST Action TD1402 - Multifunctional Nanoparticles for Magnetic Hyperthermia and Indirect Radiation Therapy (RADIOMAG)

University College London, Robert building 309 / Malet Place Eng 1.02
21st - 22nd of April 2016

The particle's physico-chemical properties are extremely important for their specific applications. Characterization is done by using a variety of different techniques depending on the application of interest. Particle characterization is a still unsolved problem at nano-regulation level. For particle characterization should be a characterization strategy or protocol developed and kept. Currently several organizations/companies are dealing with regulatory testing of manufactured materials. Here is a list of some of them. More information and links are at the end of the document.

- *NANoREG* – A common European approach to the regulatory testing of Manufactured Nanomaterials
- *European Medicines Agency* - The Agency's *Committee for Medicinal Products for Human Use (CHMP)*
- *The Organisation for Economic Co-operation and Development (OECD)*

During the workshop many issues were discussed, several of them are reported.

- **Difference between administered and delivered dose** which occurs due to transport processes, sedimentation, diffusion, etc. The delivered dose can be calculated. http://www.pnl.gov/science/highlights/highlight.asp?id=1
- **Possibility to easy calculate delivered dose** under different conditions was given to WG members by prof. Hofmann.
- **Time dependent solubility of MNPs and toxic reaction** that can arise from cations and from particles itself.
- **Different techniques** used for **size measurements and their limitations** were also discussed:

- *Transition Electron microscopy (TEM) - appropriate method for primary particle size measurement*
- *Dynamic light scattering (DLS)* - limitation: only for monodisperse samples
- *Nanoparticle tracking analysis (NTA)* – limitation of the minimum size that can be measured
- *Small angle scattering methods* – limited access
- *Powder X-ray diffraction (XRD)*
- *Coulter counter, and many others*

- Each technique requires **proper calibration standards** for the desired zones of interest.
- **Density measurements** of the solids– the only method which gives reproducible results is **He-Pycnometer**.
- Density of nanoparticles – protocol for determination of dispersibility of NPs (NANoREG draft version 2015).
- It was pointed out on the **difference between solubility and dispersibility of NPs.**
- Use of a common dispersion protocol (NANoREG sonication protocol).
- **Coating can prevent NPs solubility** which depends also on coating bonding.

- **Problems occurring by *in vivo* application of NPs** were discussed (protein corona, blood flow, etc.).
- For better results of *in vitro* test some **conditions were recommended** (shaking of the incubated cells, etc.).
- **Important parameter like Dosage-Metrics are still under discussion:** Deposited mass, Core size and distribution, Agglomerate size (and distribution, Density, Agglomerate density and distribution, Solubility, Coating, etc.

From *Frank Couillaud* talk: Nanoparticles from the tube to the tumour cells

NP interacts with culture cell media
- Avoid: NP degradation, NP precipitation, aggregation, High toxicity

Wait for NP entering the cells (endocytosis)

NP require some specific elements for specific endocytosis
For specific endocytosis < approx.100 nm
NP are stored in a vesicle
Most of the time, it's a nonspecific process.

Testing NP toxicity
In vitro
Cells could be placed in a magnetic field to measure heat production
Cells surviving (counting, sorting, reporter gene i.e. bioluminescence)
NP sub-cellular localization by microscopy, TEM
Simplification:
Rather simple systems
No competition with others cell populations
Static environment

In vivo
Injection in complex compartments
- Complex composition (lipids, proteins, enzymes)

Degradation, aggregation
- Blood flow
  Dilution
- Cleaning organs: kidney (< 5nm), liver
  Elimination, trapping
- Cells (macrophages)
  Phagocytosis

Make NP furtive (coated NP with dextran, PEG, ……)
To avoid phagocytosis by macrophages
To increase plasmatic half-life of NP in the circulation

NP accumulate in the tumour
Only a few percentage of NP reach the tumour

Specific endocytosis in cancer cells requires targeting agents
- Receptor / Clathrin-mediated endocytosis

But targeting agents may affect NP furtivity (in the blood and in the tumour)

Challenge for chemist: to add targeting agents without affecting furtivity

Conclusion
Not easy
but
-Some targeting agents do the job
-Some compartments could be shunted (local injection)
-Alternative strategies (cells as vehicles, physical disruption of biologicals barriers, ...)
-So many different cancer types (tumours properties)
-*In vivo* imaging can help

Discussion
The following issues were discussed:

- ***In vivo* and *in vitro* are completely different systems. By testing can be obtained absolutely different results. Makes it sense to do *in vitro* tests where the results can be completely different from *in vivo* results?**

  - *In vitro* testing is very important from ethical and many other reasons. There are rules, that is necessary to test all new chemical and pharmaceutical products on cultured cells before humans, to study mechanisms, study of the entering the cells, to evaluate toxicity, to estimate of the potential risk and to evaluate any potential hazard associated with its exposure to human.
  - It is type of so-called pre-screening cell test and gives a possibility to do large screening.

- **Is it possible to do 3D magnetic driving of the magnetic nanoparticles (MNPs)?**
  - Solution, how to deliver therapeutic agent close to cancer cells *in vivo* can be
    - Focused implanted magnet
    - Magnetic targeting
    - Injection to the tumour

    There are so many types of the cancer. It can work for ones, but not for the others, e. g. prostate cancer is a specific problem where imaging is really difficult. It is needed to cooperate with clinicians (what they want or need).

- **How efficient is "human" system compared to mice?**
  - Human is more complex system, the molecules can be different, some molecules are not produced by tumour directly but by the tumour microenvironment.
  - To examine response to therapy human tumour cells are transplanted to mice what enable us to get closer to the conditions in the body.

- **Nuclear medicine**
- **Cryotherapy**
- **Administered dose is different from delivered dose due to particle aggregation and sedimentation processes. Currently there are no method to describe or control process in the body. Aggregation changes the particle properties for hyperthermia also.**
- **How to estimate density of aggregates?**
  - time dependence of centrifugation process and measuring to properties of the pellet
- **What is more suitable for cancer treatment intratumour injection or intravenous injection?**
  - It depends on the cancer type.

- **Is it possible to kill metastasis by heating by means of magnetic fluid hyperthermia (MFH)?**
  - It can be achievable.

- **Activated immune response in human** can help us to treat cancer. Two months ago was approved genetic activation of immune response in the body…- immunogenicity for antibody molecules).

- It was discussed also **type of magnetic material** that is more suitable for biomedical applications.
- It was discussed why is needed hyperthermia when T-lymphocytes reach the tumour, applying RF-field for drug release.
- **Discussion about biocompatibility of Co** based nanoparticles and their toxicity. They can be applied for prolonging of life in the last stage of the cancer.
- Questions and **problems concerning the NPs preparation** challenges were discussed.
- It was discussed the question about **correctness to dilute the samples** for toxicity tests. There is different concentration used for DLS measurement, hyperthermia and applications in mice and human.
- **Production of the liposomes** are difficult due to low reproducibility, randomness, self-assembly process.

Comment: The story of hyperthermia with small particles in AC magnetic fields started in the late 1950s, a lot of studies were done, multiple research was carried out, some results were achieved, e.g. prof. Dr. Andreas Jordan, who received European approval for his medical product with nanoparticles. It seems (to Ivo) that we (this COST Radiomag action) are starting again from preparation, characterization and searching for possible medical application. It could be useful to cooperate with people, who had gone through this process/development and succeed. (What are the tasks to be achieved for this COST action?)

Recommendations/tasks for the next period

- To get/obtain/buy **ISO International Standards**
- **Communication between COST Radiomag and NANoREG** company.
- Standardized measurements of physicochemical parameters for *in vitro* tests are not available. **It is necessary to work out standard protocol to access toxicity collectively** (similar like ring test).

- It is **needed to cooperate with clinicians** (what they want or need).

List of several organisations that deals with regulatory testing, scientific guidelines, International Standards that can be very helpful for RADIOMAG members.

- **NANoREG** – A common European approach to the regulatory testing of Manufactured Nanomaterials http://www.nanoreg.eu/

- **European Medicines Agency** - The Agency's *Committee for Medicinal Products for Human Use* (CHMP) gives us overview of comments received on Reflection paper on the data requirements **for intravenous iron-based nano-colloidal products** with reference to an innovator medicinal product. CHMP prepares **scientific guidelines** in consultation with regulatory authorities in the European Union (EU) Member States, to help applicants prepare marketing-authorisation applications for human medicines.
  http://www.ema.europa.eu/docs/en_GB/document_library/Overview_of_comments/2015/03/WC500184921.pdf

- **The Organisation for Economic Co-operation and Development (OECD)** ttp://www.oecd.org/

- **International Organisation for Standardisation (ISO)**
  http://www.iso.org/iso/home.html

  - Is an independent, non-governmental international organization with a membership of 161 national standards bodies. Through its members, it brings together experts to share knowledge and develop voluntary, consensus-based, market relevant International Standards that support innovation and provide solutions to global challenges.
  - Develops and publishes International Standards.
  - Several ISO examples useful for particle size measurements by Photon Correlation Spectroscopy:
    - **International Standard ISO13321** Methods for Determination of Particle Size Distribution Part 8: Photon Correlation Spectroscopy, International Organisation for Standardisation (ISO) 1996.
    - **International Standard ISO22412** Particle Size Analysis - Dynamic Light Scattering, International Organisation for Standardisation (ISO) 2008.
    - **There are many others useful standard**