COST Office
Avenue Louise 149
1050 Brussels, Belgium
t: +32 (0)2 533 3800
f: +32 (0)2 533 3890
office@cost.eu

www.cost.eu

Košice, 20.02.2017

Subject | Minutes of the WG1 and WG3 subgroup meeting
COST Action TD1402 (RADIOMAG)
"Ferrofluid cytotoxicity ring test with SOP for NP fabrication"

Congress center ACADEMIA, Stará Lesná (Slovakia)
13th and 14th of February 2017

1st Day 13th of February 2017
Morning

1. Welcome to participants

The meeting started at 9:00 AM.

Present are: Nguyen T.K. Thanh, Eleni Efthimiadou, Sylvie Begin, Daniel Horak, Marijana Mionic Ebersold, Tung Le, Raquel Rodrigues, Vlasta Zavisova
Other guests: Alena Jurikova, Martina Koneracka, Peter Kopcansky, Martina Kubovcikova, Matus Molcan, Andrej Musatov, Ivo Safarik, Katarina Siposova, Milan Timko

The meeting was opened by Vlasta Zavisova and Nguyen T.K.Thanh. The participants were welcomed by Peter Kopcansky (local organiser) at the Congress center ACADEMIA, Stará Lesná.

2. Meeting content

Current work and new results – lectures

- **Peter Kopčanský:** Research at Institute of Experimental Physics Slovak Academy of Sciences.
- **Sylvie Begin-Colin:** Magnetism engineered nanoparticles towards innovative biocolloids combining MRI and magnetic hyperthermia properties

Sylvie Begin-Colin showed a concept combining a dendritic coating with magnetic IONPs designed for MRI and magnetic hyperthermia. Dendronized iron oxide nanoparticles (IONPs) were demonstrated to induce any cytotoxicity. *In vivo* and *in vitro* MRI measurements showed that their contrast enhancement properties were higher than those obtained with commercial polymer-coated nanoparticles (NPs). Moreover, both types of dendronized NPs were eliminated by urinary and hepatobiliary pathways without unspecific uptake especially in the reticuloendothelial system (RES) organs and in the lungs. The design of dendronized NPs was further improved to obtain theranostic nano-objects by adjusting the morphology and the composition (cubic and octopod shape and core-shell) of the inorganic magnetic core and by designing multifonctionalized dendrons. These NPs were found suitable to combine imaging and therapy

by hyperthermia. Finally, these dendronized NPs bearing melanin vectors were demonstrated very suitable to specifically target *in vivo* tumoral cells.

Skype call with Simo Spassov

Simo was asked to inform participants about results of SAR ring tests and conclusions of WG2 meeting held in Zaragoza, 2017.

Main issue from the skype call:

- Two SAR ring tests had been evaluated and the stability of prepared samples was checked by Dynamic Light Scattering (DLS) method

Propositions what to do next:

- Recalculation of SAR values by different method
- Used different field values
- Measured samples from12/22 labs
- Planned 3rd SAR ring test in March (samples No. 4 and 6)
- SAR dependency on field intensity and frequencies
- Uwe Steinhoff is currently occupied with a relative calibration method with metal balls
- Detailed information can by find at Minutes of the WG2 SAR sub-group Meeting of COST held in University of Zaragoza, Zaragoza (Spain), 9th -10th of February 2017
- Further details are given in the Zaragoza meeting minutes

Lunch between 1 PM and 2 PM.

1st Day – Afternoon

Cytotoxicity ring test – Brainstorming session, WG1/WG3 Disscussion
Recommendation for nanoparticles fabrication, testing, and requirements for NPs concentration.

In discussion, individual participants exchanged their experience gained in laboratories in the field of nanoparticles preparation, characterization, cytotoxicity testing and magnetic hyperthermia tests. One of the participants shared their experience with *in vitro* hyperthermia tests. More than hundred time vs temperature curves were acquired by different operators in different laboratories and with 5 different MFH test devices on the samples from the same batch. Obtained SAR values range from 100 – 500 W/g. Most of them were in the range 420-520 W/g. One of the devices gave always value round 100 W/g. Mapping of magnetic field inside the coil of this device showed that the field values declared by the produce were only met in an area of 3 mm in height and 2.5 mm in width, which is unacceptable.
Participants performed magnetic hyperthermia measurements were invited to take part in the ring tests organized by the WG2 SAR subgroup.

Concentration of magnetic nanoparticles 10 mg/ml and less was recommended for the first fast indicative measurement to assess whether the prepared sample is appropriate for magnetic hyperthermia or not.

Next point of discussion concerned the difference in nanoparticles concentration used for cytoxicity testing (that is usually very low) and nanoparticles concentration used for hyperthermia measurements (that is usually several orders of magnitude higher). The development of new types of more sensitive nanoparticles with better thermal effect could help to solve out this problem.

Planning magnetic fluid cytotoxicity ring test

Planed cytotoxicity ring test was discussed next. There was expressed an opinion that it is not possible to arrange the same conditions for cytotoxicity testing. There are too many things that should be taken into account. It would be very difficult to keep the same conditions for sample handling. For example, sample can be totally different if it was shipped by air (located in the luggage compartment frozen) or on land and therefore the cytotoxicity results can be different. The participants discussed about the difficulties of SAR tests due to cell variations even in the same cell line between different labs.

Important parameters to consider for nanoparticles – cell interactions (WG1-WG3) were discussed and individual tasks were dealt with for preparation of common Radiomag review paper as follows:

NPs

- Size, size distribution, shape, surface chemistry (charge, hydrophobicity, chemical composition), concentration: **Nguyen TK Thanh, Eleni K. Efthimiadou** (size, shape)

- Synthesis routes: precursors (impurity), ligands, characterization of ligands, completeness of the exchange: **Sylvie**
- Storage conditions of NPs, posting: **Marijana Mionic Ebersold**

PROTEIN CORONA

- Cell culture media: **Eleni, Frank**
- Cell types: breast cancer cells: **Eleni**
- Incubation times, temperature: **Eleni**
- Passage of cells (resistance): **Eleni**
- Cytotoxicy assays: absorbance and fluorescent base assays
- How do you express cytotoxicity (is it mass, or mols, or surface area), concentration: **Marijana, Sofia Lima**
- Reproducibility of test: **Sofia Lima**
- Hyperthermia induced toxicity: **Every one wish to contribute at Cost**

The first meeting day finished around 6 PM.

Tuesday 14 February 2017

2nd Day – Morning

The meeting started at 9 AM by the following lectures concerned current work and new results

- **Raquel O. Rodrigues:** Multifunctional graphene-based yolk-shell magnetic nanoparticles assessed as pH-dependent controlled release of anticancer drugs
- **Nguyen T. K. Thanh:** Doxorubicin loaded dual pH- and thermo-sensitive magnetic nanosystem for combined hyperthermia and controlled drug delivery applications
- **Marijana Mionic Ebersold:** Reinventing iron oxide nanoparticles for superior MRI detection and hyperthermia treatment
- **Eleni K. Efthimiadou:** Advanced theranostic NPs based on magnetic materials: In vitro and in vivo applications

Raquel O. Rodrigues informed participants about developed **graphene-based yolk-shell MNPs**,that exhibit **exceptional characteristics** to be applied as **drug nanocarrier system**, namely:

- good loading capacity;
- high pH-sensitivity;

- high magnetic saturation;
- superparamagnetic behaviour,
- biocompatibility.

Additionally, the combination of these parameters also indicates a high potentiality to couple these nanosystems with other biomedical applications, such as magnetic hyperthermia. This combined strategy could represent an important step in cancer treatment.

Nguyen TK Thanh informed participants about developed a novel nanotherapeutic composed of an iron oxide core and a thermo-responsive polymer shell. The magnetic nanocomposite (MNC) allow for a triggered release of drugs as a consequence of hyperthermia and tumor acidic pH, through breakage of pH and heat labile Schiff base bonds that bind the drug molecules to the polymer. Iron oxide NPs synthesized by a microwave-assisted co-precipitation method were functionalized with the thermo-responsive polymer via a silanisation reaction. After fully characterizing the MNCs, their heating performances were evaluated. Doxorubicin (dox) was loaded into the nanosystem via formation of imine bonds between the amine group of dox and the aldehyde group of the polymer, and the drug release kinetics were carefully studied as a function of the pH and the temperature.
The MNPs show a superparamagnetic behavior with a saturation magnetization around 70 emu/g. The LCST of the polymer was engineered around 39 - 40 ˚C and its successful grafting on the MNP surface was confirmed by FTIR and TGA analysis, yielding MNCs with a hydrodynamic diameter around 120 nm and good colloidal stability. Their potential as nanoheaters was confirmed with an ILP > 1.0 nHm²/K. As expected, faster and higher release of drug was obtained under hyperthermia conditions and tumour acidic pH, with 85.2% of drug released after 48 h. Finally, MTT assay indicated that the MNCs show no cytotoxicity to cells even with concentrations up to 1 mg/mL.

Eleni K. Efthimiadou presented work focused on nanostructured delivery and diagnostic systems that induces specific targeting properties by exploiting the local physicochemical tumor characteristics. It is well known that cancer cells have specific physicochemical characteristics, which can be taken into consideration for the design of a broad spectrum of drug delivery systems (DDS). Some of those characteristics including the different temperature environment their susceptibility when temperature ranges between 40-43 °C where cell apoptosis is induced, the intra- and extra- cellular pH which varies from 6.0 to 6.8, for cancer cells, and 6.5 to 7.4 for normal cells respectively, (lysosomes acidic pH ranges 4-5). Additional significant factors are the overexpressed receptors on the tumor surface. Loading and release studies were carried out by using the anthracycline drug Doxorubicin and their cytotoxicity was evaluated by using the MTT assay in healthy and diseased cell lines. The highlight of this work is the in vitro and in vivo study which were performed in order to evaluate different nanostructures as for their biodistribution, pharmacokinetic and toxicity per se.

Lunch between 1 PM and 2 PM

2nd Day – Afternoon

- **Daniel Horak:** Physico-chemical characteristics, biocompatibility, and MRI applicability of novel monodisperse PEG-modified magnetic Fe3O4&SiO2 core-shell nanoparticles
- **Matus Molcan:** Magnetic Hyperthermia – possibilities and selected experimental results

Daniel Horak informed participants about availability of

- *D*-mannose-, bisphosphonate-, PLL-, PDMA-, PANI-, PEG, and SiO_2-coated γ-Fe_2O_3 or Fe_3O_4 nanoparticles;
- Particles heating under AC magnetic field;
- Cytotoxicity of the particles determined by MTT viability, respiratory burst and phagocytic activity of human leukocytes; the particles proved to be non-cytotoxic;

- Slight ROS production, mitochondrial depolarization, and activation of antioxidative defense mechanisms was observed upon internalization of Fe_3O_4@SiO_2 nanoparticles;
- Fe_3O_4@SiO_2-PEG nanoparticles were not internalized by mNSCs, in contrast to other particles;
- PLL@γ-Fe_2O_3 particles are suitable for general *in vivo* cell labeling and tracking as they are intensively internalized and provide high MRI contrast; for example, reverse transport of cholesterol was confirmed by MRI using these particles, which is important for prediction of atherosclerosis;
- PDMA@γ-Fe_2O_3 nanoparticles exhibited antitumor and antimetastatic activity.

Matus Molcan informed about preparation and isolation of magnetosomes and ability to modify their properties by using different synthesis conditions during cultivation process. Then introduced set up used for calorimetry study of different magnetic materials studied in their laboratory. SAR values as well as the presence of biological membranes showed that bacterial nanoparticles are promising material for magnetic hyperthermia but on the other hand production of big amount of this type of nanoparticles would be really difficult.

Brainstorming and WG1/WG3 disscussion

Marijana recommended to Radiomag members to use maghemite instead of magnetite for the next experiment because of absence of Fe(II) in γ-Fe2O3, since Fe(II) is inducing oxidative stress.

Marijana informed participants about suitable coil size for using of magnetic hyperthermia in clinical testing. Comertial devices usualy has coil diameter so small that almost no part of human body can be treated in it. There was recommended to focus on designing of magnetic hyperthermia equipment with dimensions suitable for human body.
Nanoreg will publish shortly regulatory protocols about nanoparticles production and characterization that could be usefull for RADIOMAG members.

SOPs which they currently have (come out from few EU and/or Swiss projects):

Name:
- Apoptosis and necrosis
- Flowchart nano specific risk assessment
- Human Lung Cell transformation assay
- LAL Assay for Nanoparticles
- LDH Cytotoxicity Assay
- Methodology for sedimented dose determination with CLS and Pixe Techniques
- NaNoReg-ECOTOX Dispersion SOP (0)
- NRCWE SOP for measurement of hydrodynamic Size-Distribution and Dispersion Stability by Dynamic Light Scattering (DLS)
- PhD De Temmerman Hardback
- Probe Sonicator Calibration SOP_ecotoxicological testing_V3_jan2015
- Production of proinflammatory cytokines
- Prospect dispersion protocol
- Protocol for particle size determination of a given MNM by the centrifuge liquid sedimentation (CLS) technique
- Protocol for the Preparation of aqueous dispersions of carbon nanotubes (CNTs)
- Protocol to quantify the concentration of a given MNM in cell culture media with the Particle-Inducted X-ray Emission (PIXE) technique
- Reaction Oxygen Species Detection

- SOP – Electron microscopic image analysis of nanomaterials
- SOP for determination of Dispersibility from Delivery 2.9(1)
- SOP for determination of Dispersibility from Delivery 2.9
- SOP - Preparation of EM-grids containing a representative sample of a dispersed NM
- SOP – Qualitative description of NM based on TEM micrographs
- SOP – Transmission electron microscopic imaging of nanomaterials
- SOP for DLS measurements using MALVERN NANO ZS_2016-11-07
- SOP for effective density from Delivery 2.9
- SOP for Isoelectric point determination from D2 9
- SOP for test item preparation and comparability of results during in vitro testing
- SOP for true density from Delivery 2.9
- SOP for Water Solubility using the flask method from D2 9
- SOP Electron microscopic image analysis of primary particles in aggregated nanomaterials
- SOP for true density from Delivery 202.9
- Tackling confounding factors in nanomaterial hazard assessment
- Technical guidance Document for Environmental Exposure Characterisation final
- SOP for true density from Delivery 202.9
- Tackling confounding factors in nanomaterial hazard assessment
- Technical guidance Document for Environmental Exposure Characterisation final

A web link regarding SOPs, **Nanoreg guidelines**:
http://www.nanoreg.eu/media-and-downloads/factsheets-of-nanoreg-output

3. Bilbao meeting

Nguyen TK Thanh will inform RADIOMAG members about conclusions from WG1/WG3 meeting held in Slovakia at next annual meeting.

4. Closing

Participants thanked to COST office for opportunity to attend such marvellous meeting, to grant holder Simo Spassov and to local organizers for hospitality and meeting organization. Organizers thank to all participants for their contributions and fruitful discussions.

The meeting was closed around 6 PM.

List of Annexes

Annex 1: Signed COST Attendance list