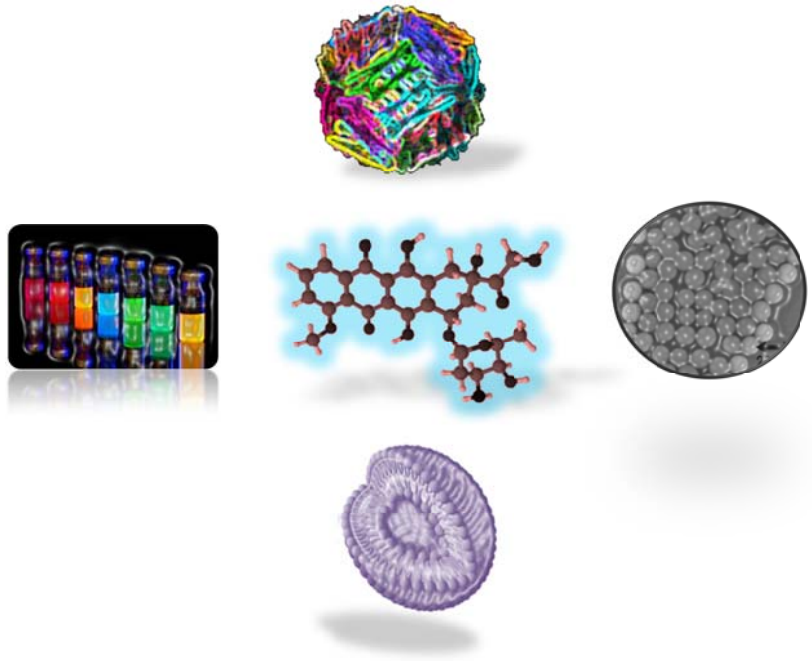
CZ.1.07/2.4.00/31.0023 NanoBioMetalNet
Partnerská síť centra excelentního bionanotechnologického výzkumu
Laboratoř Metalomiky a Nanotechnologií, laboratoř nanoelektrochemie

Invites you to the training:

NANOMATERIALS FOR MEDICAL APPLICATION

Annotation

The biological and medical research communities have exploited the unique properties of nanomaterials for various applications (e.g., contrast agents for cell imaging and therapeutics for treating cancer). Terms such as biomedical nanotechnology, nanobiotechnology, and nanomedicine are used to describe this hybrid field. Functionalities can be added to nanomaterials by interfacing them with biological molecules or structures. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications.



Thus far, the integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles.

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Program

8:30 Opening ceremony – Doc. RNDr. Vojtěch Adam, Ph.D.
Mgr. Markéta Vaculovičová, Ph.D.

9:00 – 9:45

Real-Time Sensing of Doxorubicin in an Isolated Rat Heart

Blazkova I., Vaculovicova M., Novakova M., Adam V., Kizek R.

Doxorubixin is a highly effective and widely used antracycline antibiotic cytostatic drug used to treat numerous types of tumor diseases, but the cardiotoxic effect significantly limits its application. Doxorubicin has hot great fluorescence properties what can be used to its detection. The detection of the fluorescence of the therapeutics in organisms is limited by the thickness of the tissue the light need to penetrate. An alternative way for increasing the sensitivity of this type of imaging is the elimination of surrounding tissue, leading to *ex vivo* analysis under simulated conditions (i.e. perfusion system for isolated heart). The aim of this study was the combination of perfusion system with the fluorescence *in vivo* imaging system to observe the fluorescence compounds in beating heart.
The study proposes the application of *in vivo* imaging system for fluorescence *ex vivo* analysis of rat heart from the doxorubicin administered rat. The miniaturized Langendorff perfusion system was used. The isolated heart was supply by oxygenated Tyrode solution (37 °C) to ensure the heart beating and nutrition. This arrangement enabled the detection of doxorubicin in the ex vivo heart. The detection concentration was 1 μg of doxorubicin in the heart after the intraperitoneal application of 4 mg doxorubicin.

9:45 – 10:30

The effect of heavy metal ions on *staphylococcus aureus*

Chudobova D., Dostalova S., Ruttkay-Nedecky, B., Merlos Rodrigo M. A., Adam V., Kizek R.

Our objective was to determine the effect of heavy metal ions on resistant strains of Gram-positive bacterial strain of *Staphylococcus aureus* using mass spectrometry. The resistant strains of *S. aureus* were prepared using the nitrate solutions of metals (Ag, Cu, Cd, Zn and Pb). MALDI-TOF mass spectrometry was used for observation the changes in the protein

composition in the cell wall and also for the determination and identification of the strains using the database MALDI Biotyper. Results obtained from analysis with resistant strains were compared with sensitive control strain of *S. aureus*. We observed alterations in *S. aureus* protein composition pointing at resistance development under influence of heavy metals ions. Our results develop the possible option of analysis of resistant strains and may serve as a support for the monitoring of changes in genetic information in resistant strains.

10:30 – 11:15

Liposomes as drug carriers and their characterization using different analytical methods

Guran R., Kominkova M., Kopel P., Chudobova D., Zitka O., Adam V., Kizek R.

The physicochemical properties of liposomes are significantly affected by the composition of phospholipid bilayer; differences in composition allow the use of liposomes for analytical purposes and for therapeutic purposes. One of the most used components of phospholipid bilayer is cholesterol. Its concentration plays a significant role in the behavior of liposomes.

This study points to changes in the properties of liposomes and its influence on encapsulated doxorubicin according to the content of cholesterol in the phospholipid bilayer. The influence of SDS addition to liposomal variants was also evaluated. Three variants of liposomes differing in various concentrations of cholesterol were assessed.

Firstly, the toxicity of all types of liposomal doxorubicin was evaluated and it was found that the content of cholesterol increases the IC_{50} values of encapsulated doxorubicin in liposome. The highest concentration of cholesterol in liposome increased the IC_{50} value even four times compared to liposomes without cholesterol.

Secondly, the new approach to compare the influence of different variants of liposomes on detection of carried doxorubicin was used using the electrochemical detection with construction of differential hydrodynamic voltammograms.

11:15 – 12:00

3D Chip as a tool for isolation and detection of influenza vaccine Hemagglutinin

Krejcova L., Hynek D., Kopel P, Adam V., Kizek R.

The aim of this study was an isolation and detection of influenza antigen using 3D fabricated chip. Surface of influenza virion is equipped with two antigens: hemagglutinin (HA) and neuraminidase (NA), which are responsible for virus life cycle and interaction with host cell. In this study, vaccine HA, labeled by quantum dots (QDs) was used for better specification. The 3D chip assay was divided in two parts: paramagnetic particles (MPs) based isolation and electrochemical detection of isolated product. Our results show, that 3D fabricated chip is useable tool for MPs based isolation and electrochemical detection influenza hemagglutinin.

12:00 – 13:30

LUNCH BREAK

13:30 – 14:15

Apoferritin as a targeted drug delivery system

Dostalova, S., Konecna, R., Blazkova, I., Vaculovicova, M., Kopel, P., Krizkova, S., Vaculovic, T., Adam, V., Kizek, R.

Conventional cancer treatment often effects normal cells and has many side effects. These can be addressed by the use of nanomedicine, especially its platform nanotransporters, in which cytostatic drug can be encapsulated. These nanotransporters can be dispersed in tumors through relatively large pores in tumor blood vessels but not in normal blood vessels. The nanotransporters can be coupled with antibodies, thus allowing targeted delivery of drugs. For this coupling we chose the method using linker, small peptide that interacts with Fc region of IgG antibodies, presenting the antigen binding site facing out.

The aim of this experiment was to create, characterize and test a nanotransporter based on apoferritin nanocage with encapsulated doxorubicin, modified with specific antibody. As a linker, heptapeptide HWRGWVC was used. Cysteine has known affinity towards gold, two methods of apoferritin surface modification were proposed and better results were obtained with apoferritin modified with gold nanoparticles. The presence of gold on apoferritin was proved by separation in polyacrylamide gel electrophoresis (PAGE), followed by inductively coupled plasma mass spectrometric (ICP-MS) measurement. Enzyme-linked immunosorbent assay (ELISA) was used to prove the apoferritin ability to specifically bind to target cells. Apoferritin retained its ability to open and release doxorubicin in low pH. This is very convenient, since there is lower pH in tumors due to hypoxemia.

14:15 – 15:00

Utilization of the iron nanoparticles for heavy metal removal from the environment

Kremplova M., Fialova D., Hynek D., Adam V., Kizek R.

The pollution of the aquatic ecosystem by heavy metals is one of the global environmental problems, which deals with many world´s institutions. The aim of this work was to design a process for the isolation of heavy metals in surface and waste water. There were used the basic heavy metals like cadmium, lead and copper, which have been isolated from aqueous solutions using iron nanoparticles Fe_2O_3. Electrochemical methods of differential pulse voltammetry and linear sweep voltammetry were used for the heavy metals detection. After a one day interaction of heavy metal solutions with nanoparticles there was monitored 100% adsorption of cadmium, lead and copper on the Fe_2O_3 surface.

15:00 – 15:45

Fluorescence properties of quantum dots

Smerkova K., Blazkova I., Chudobova, D., Vaculovicova M., Kopel P., Adam V.,Kizek R.

The aim of this study was the synthesis of CdTe quantum dots (QDs) and study of their fluorescence properties and their potential for the use in the bioimaging. QDs are small semiconductor nanoparticles (1 – 20 nm), which can be used in the imaging instead of the organic labels. The CdTe QDs were synthesized by microwave synthesis in an aqueous solution. As the source of telluride, Na_2TeO_3 was used, and as reduction agent, sodium borohydride was applied. Quantum dots were stabilized by mercaptosuccinic acid (MSA). According to reaction conditions (temperature: 50 – 130 °C), size of prepared quantum dots can be tuned. Synthesized QDs had got very good fluorescence properties and were used for the cell labeling. The QDs penetrated into the cells and stained plant cells, as well as human foreskin fibroblasts. But the changes in the cells shapes were observed, the reason could be the toxic effect of QDs, which should be more investigated.
For the usage of QDs in medicine, it is necessary to know their behavior in the tissue. We investigated the behavior of QDs in the chicken breast muscle tissue. After the direct injection of QDs into the muscle tissue, sufficient spreading of QDs in the tissue was observed and a significant linear increase of the fluorescence intensity of QDs with applied volume was

determined. To detect the limiting depth for the signal detection, the tubes filled with different QDs was inserted into the different depths of the tissue. The intensity of the fluorescence of QDs depended on the size of QDs, therefore red QDs was possible to detect the most deeply (10 mm). Using the different emission filters, it is possible to distinguish between the different QDs. It enables the use QDs in the simultaneously labeling of different structures in the cells or the organisms.

15:45 – 16:00

COFFEE BREAK

16:00 – 16:45
The use of nanotechnology as modern tools to treat infections caused by multiresistant bacteria strains

Sklenar M., Chudobova D., Ruttkay-Nedecky, B., Kryštofová O., Kopel P., Adam V., Kizek R.
This work focuses on development of antimicrobial complex substances, suitable to cover vascular implants with the secondary use in transplantation surgery. This work also presents a comparison of the effects of used nanomaterials against ordinary substances. The formation of complexes took place between silver nanoparticles, silver ions and the polymer substances (hyaluronic acid, collagen and chitosan). The ability of complex formation of these substances was studied using electrochemistry and spectrophotometry. Bactericidal effect of these compounds was determined by growth-curve methods and inhibition zones on a bacterial culture Staphylococcus aureus. The viability of eukaryotic cells in straight confrontation with tested substances was observed using the MTT test. According to the data obtained, the complex of silver nanoparticles with chitosan was evaluated as the best substance to ensure antimicrobial behaviour of vascular implants.

16:45 – 17:30

Electrochemical detection of the interaction of quantum dots with metalothionein

Tmejova, K., Kremplova, M. , Jarosova, M. , Hynek, D., Nejdl, L., Kopel, P., Adam, V., Kizek, R.

In the last period the interest of scientists is focused on nanoparticles (quantum dots, nanotubes, nanowires). Quantum dots (QDs) are widely studied. They can emit light radiation and from this reason they could be used like a fluorescence label for in vivo imaging. QDs can also bind proteins by unspecific binding. One from the most important protein in human body is metallothionein (MT), small cysteine-rich protein, which is responsible for binding of heavy metals, for accumulation of Zn, protection of cells to oxidative stress and it participates in the regulation of expression a number of major genes and enters to oxidative-reductive balance in a cell. From these reasons the interaction of MT with QD could play the important role at using QD in living organism. The study of this interaction is possible due to the electroactivity of both integrated components by electrochemical methods.
The aim of this experiment was the study complexes MT-QDs created during the interaction of metallothionein with CuS QDs. Complexes determined by peaks Cat1, Cat2, RS2Co, Y and X were investigated in Brdicka´s solution by the differential pulse voltammetry on mercury electrode. The used interaction time was: 0 and 480 s; 30, 60, 90 min, and 2, 3, 4, 5 and 6 hrs. Brdicka´s solution was used as an electrolyte

Friday 15th November 2013, start at 09:00
Department of Chemistry and Biochemistry, Mendel University in Brno
Contact: kizek@sci.muni.cz