Contribution ID: **199** Type: **Oral Presentations**

3D Mapping of Folate-Functionalized Silica Nanoparticles in Cells via Combined Fluorescence, Dark Field, and Hyperspectral Microscopy

Thursday 10 July 2025 12:45 (15 minutes)

The development of efficient drug delivery systems is important in modern medicine to improve therapeutic efficacy while minimizing side effects. Among various nanocarriers, mesoporous silica nanoparticles (MSNs) have emerged as versatile platforms due to their high surface area, tunable pore size, and capacity for surface modification. Surface functionalization of MSNs with targeting ligands, such as folic acid, enhances their specificity by exploiting receptor-mediated endocytosis –particularly in cancer cells that overexpress folate receptors.

Targeted delivery becomes especially valuable in the administration of potent chemotherapeutics like Irinotecan (Iri), a topoisomerase I inhibitor widely used in colorectal cancer treatment. However, its systemic toxicity poses significant challenges. By encapsulating Iri within MSNs and decorating them with folate, it is possible to achieve selective cytotoxicity toward tumor cells while sparing healthy tissue.

This in vitro study aims to assess both the cytotoxic potential and intracellular mapping of folate-functionalized, Iri-loaded MSNs. By combining advanced imaging techniques, including hyperspectral and fluorescence-based enhanced dark field microscopy, with quantitative image analysis, this research investigates how nanoparticle surface chemistry influences cellular uptake and subcellular localization in cancerous versus non-cancerous cells.

Caco-2 (human colon adenocarcinoma) and NIH3T3 (mouse fibroblast) cells were used as cancerous and non-cancerous models, respectively. After 24 hours of seeding, the cells were incubated with the folate-functionalized, Iri-loaded MSNs for 24 or 48 hours continuously (24h+, 48h+). A separate experimental category was assigned to a discontinuous incubation with the nanoparticles, after the first 24h of incubation the culture medium containing MSNs being replaced with physiological culture medium (24h+/-). Cell viability was measured at mitochondrial level using the colorimetric MTS assay (formazan based).

Microscopy analyses were conducted using enhanced dark field microscopy (eDFM) from Cytoviva®, operating in hyperspectral imaging (HSI) and fluorescence imaging modes. Fluorescence imaging included cytoskeletal staining with AlexaFluor488 Phalloidin and nuclear staining with DAPI, enabling 3D reconstruction of cellular structures. For hyperspectral analysis, non-stained Caco-2 cells were examined and spectral profiles at pixel level were generated. Image processing was done using Cytoviva® software and custom MATLAB routines leading to 3D cell reconstructions and quantifications of MSN localization in nuclear and cytoplasmic regions.

Folate-functionalized MSNs demonstrated significantly enhanced cytotoxicity compared to non-functionalized controls, with greater effects observed in Caco-2 cells than in NIH3T3 cells. This outcome aligns with the known overexpression of folate receptors in cancer cells. Hyperspectral and fluorescence microscopy revealed that folate conjugation facilitated greater MSN uptake and preferential localization near the nucleus. The custom image analysis pipeline enabled detailed 3D visualization and quantification of MSNs within cellular compartments.

This study demonstrates that folate-functionalized, non-fluorescent MSNs significantly enhance the delivery and cytotoxicity of Irinotecan, particularly in human colon adenocarcinoma cells. The application of advanced microscopy techniques, combined with a novel processing approach for Z-stack images, enabled quantitative evaluation of nanoparticle uptake and intracellular distribution. These findings highlight the potential of folate-targeted MSNs for efficient and selective cancer therapy.

Acknowledgments: This work was supported by projects PN-III-P2-2.1-PED2019 (contract no. 525PED/2020) and Project No. P_36_611, MySMIS code 107066.

Primary author: MICLEA, Luminita Claudia (Biophysics and Cellular Biotechnology Department, Excellence Center for Research in Biophysics and Cellular Biotechnology, Faculty of Medicine, University of Medicine and Pharmacy Carol Davila, 8 Eroii Sanitari Blvd., Bucharest, 050474, Romania)

Co-authors: SANDU, Ana Maria (CAMPUS Research Center, Doctoral School of Electrical Engineering, University "Politehnica"of Bucharest, 313 Splaiul Independentei, Bucharest, 060042, Romania); BREZOIU, Ana-Maria (Department of Inorganic Chemistry, Physical-Chemistry & Electrochemistry, Faculty of Applied Chemistry and

Materials Science, University "Politehnica"of Bucharest, 1-7 Polizu st., 11061, Bucharest, Romania); MATEI, Cristian (Department of Inorganic Chemistry, Physical-Chemistry & Electrochemistry, Faculty of Applied Chemistry and Materials Science, University "Politehnica"of Bucharest, 1-7 Polizu st., 11061, Bucharest, Romania); BERGER, Daniela (Department of Inorganic Chemistry, Physical-Chemistry & Electrochemistry, Faculty of Applied Chemistry and Materials Science, University "Politehnica"of Bucharest, 1-7 Polizu st., 11061, Bucharest, Romania); MOISESCU, Mihaela Georgeta (Biophysics and Cellular Biotechnology Department, Excellence Center for Research in Biophysics and Cellular Biotechnology, Faculty of Medicine, University of Medicine and Pharmacy Carol Davila, 8 Eroii Sanitari Blvd., Bucharest, 050474, Romania); MIHAILESCU, Mona (Digital Holography Imaging and Processing Laboratory, Physics Department, Faculty of Applied Sciences, University "Politehnica" of Bucharest, 313 Splaiul Independentei, Bucharest, 060042, Romania); TARBA, Nicolae (Physics Department, Faculty of Applied Sciences, Doctoral School of Automatic Control and Computers, University "Politehnica"of Bucharest, 313 Splaiul Independentei, Bucharest, 060042, Romania); MITRAN, Raul-Augustin ("Ilie Murgulescu"Institute of Physical-Chemistry, Romanian Academy, 202 Splaiul Indepedenței, Bucharest, 060021, Romania); SAVOPOL, Tudor (Biophysics and Cellular Biotechnology Department, Excellence Center for Research in Biophysics and Cellular Biotechnology, Faculty of Medicine, University of Medicine and Pharmacy Carol Davila, 8 Eroii Sanitari Blvd., Bucharest, 050474, Romania)

Presenter: MICLEA, Luminita Claudia (Biophysics and Cellular Biotechnology Department, Excellence Center for Research in Biophysics and Cellular Biotechnology, Faculty of Medicine, University of Medicine and Pharmacy Carol Davila, 8 Eroii Sanitari Blvd., Bucharest, 050474, Romania)

Session Classification: Biophysics, Life Sciences, Medical Physics

Track Classification: S02 –Biophysics, Life Sciences, Medical Physics