UNIVERSITÀ DI TORINO

Dipartimento di Fisica

Nanomaterials for Industry and Sustainability

NIS COLLOQUIUM
nBIO-MED

Prospects and challenges of nanomaterial application in the BIO-MEDical field

19 April 2024

Aula Castagnoli - Dip. di Fisica

Via Pietro Giuria, 1 - Torino

Attendance is free but registration is required before 18/04/24

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Program

9.30	**Opening**
9.40	Andrea Candini (ISOF – CNR)
10.15	Martin Falk (Institute of Biophysics)
10.50	Caterina Merla (ENEA)
11.25	**Coffee break**
11.45	Pietro Aprà (INFN/Università di Torino)
12.20	Alessandro Barge (Università di Torino)
12.55	**Closing**

Andrea Candini

Andrea Candini is Senior Researcher at the Istituto Sintesi Organica e Fotoreattività (ISOF – CNR), Bologna, where he is responsible of the Advanced Microscopy and Scanning Probes Laboratory. AC graduated and got his PhD (2007) in Physics at the University of Modena e Reggio Emilia, working on the experimental study of magnetic molecules, specifically on surfaces and in nanodevices. After postdoctoral research grants, spending also two years at CNRS Grenoble (Fr), working on the use of graphene and magnetic molecules for applications in molecular (quantum) spintronics, in 2014 AC started his independent activity, being awarded of a national grant for young researchers. He joined ISOF since 2017, working on the characterization of low dimensional materials, including graphene based devices for biosensing and stimulation, and photoactive molecules and organic compounds on thin films and surfaces. More recently AC is focusing on the optimization of nanodiamonds to use nitrogen vacancy (NV) based sensing in biosystems. AC authored > 70 papers, receiving > 2800 citations with an h-index = 27.

Carbon based nanomaterials for sensing and stimulation of biological systems: examples from nanodiamonds and graphene

Materials with sizes at the nanoscale possess unique properties that make them appealing in various fields and biology and medicine are no exception. Nanomaterials are indeed currently employed in a wide range of applications, including diagnostic and imaging, therapeutic and drug delivery, and more. In this presentation, we discuss some examples of our recent activities at the intersection between nanomaterials engineering and biology, with a particular emphasis on materials that are made of carbon.

Our main focus is on the use of nitrogen-vacancy (NV) enriched diamond nanoaprticles (Fluorescent nanodiamonds -FNDs) for sensing. NV centers combine high sensitivity to several physical quantities, such as electromagnetic fields and temperature, and nanoscale spatial resolution. FNDs are very stable, non-toxic and can be easily functionalized, making them highly attractive for imaging and (bio)sensing. By doping polyvinyl-alcohol (PVA) electrospun fibers with FNDs, we demonstrated the sensing capabilities along with an interesting light-guiding effect, with the potential to mitigate some of the current limitations in the use of FNDs for applications in biology[1]. Additionally, we showcase the use of FND based thermometry in cells loaded with metal nanoclusters, illustrating an example of employing multiple nano-sized probes for simultaneous stimulation and sensing[2].

Finally, we report the use of graphene-coated electrodes with different electrical characteristics, capable to modulate intracellular calcium signaling in primary cortical astrocytes[3].

[1] R. Styles et al., Adv. Optical Mater. 2023, 2302940

[2] E. Saracino et al., under review

[3] R. Fabbri et al., accepted for publication

Martin Falk

Martin Falk (MF) received his MSc. degree (2000) and RNDr. degree (Rerum Naturalis Doctor, 2003) in Molecular Biology and Genetics at Masaryk University in Brno (Czech Republic) and Ph.D. degree in Cell and Molecular Biology (2004) at the same university. In 2021, he was appointed Associate Professor of Genomics and Proteomics. Martin's research focuses on the biological effects of various types of ionizing radiation at the micro- and nanoscale, particularly on DNA damage and repair in the context of chromatin, radiosensitization of cancer cells and radioprotection. Martin has published more than 60 papers in impacted scientific journals and contributed to several books, including the recently published Radiobiology Textbook (Sarah Baatout, ed., 2023). He has been principal investigator, co-investigator or team member in >30 national and international projects, and participated as the Czech representative (MC member) in several EU-COST projects (RADAM, Nano-IBCT, CliniMARK, MultiChem). Martin has been a plenary, keynote or invited speaker at a number of conferences and his research efforts have been recognized with several awards, such as the Premium of Otto Wichterle for outstanding young scientists by the Czech Academy of Sciences (2009) or the Deutsche Gesellschaft für DNA-Reparaturforschung (DGDR) Award (2008). Martin currently heads the Department of Cell Biology and Radiobiology and the Laboratory of Chromatin Function, Damage and Repair at the Institute of Biophysics of the Czech Academy of Sciences. He is also an enthusiastic Associate Professor at Masaryk University and Guest Professor at the University of Heidelberg (Heidelberg, Germany) where he teaches semester courses and seminars on Radiation Biophysics, Radiobiology and Chromatin Structure & Function. MF is the current President of the Society for Radiobiology and Crisis Planning of the Czech Medical Association of J. E. Purkyně (SRKP ČLS JEP), a member of the Council of the European Radiation Research Society (ERRS) and serves as Chancellor of the ERRS in the IARR (International Association for Radiation Research). He was repeatedly elected a member of the Academic Assembly of the Czech Academy of Sciences (CAS) and an expert of the CAS in the field of radiobiology. On behalf of the SRKP ČLS JEP, Martin and his wife Iva are organizing the 18th International Congress for Radiation Research (ICRR2027) in Brno, Czech Republic, from 20-24 June 2027, to which you are all cordially invited!

Persistent controversy with the mechanism of metal nanoparticle-mediated radiosensitization of cancer cells

Initial clinical studies have shown that metal nanoparticles (NPs) could represent a promising new tool in radiotherapy because of their ability to sensitize tumor cells to the effects of ionizing radiation. However, the mechanism of radiosensitization by NPs remains unclear and is the subject of intense controversy. Consistent with the central role of the DNA molecule in killing (tumor) cells with radiation, it was originally thought that irradiated NPs radiosensitize cells by emitting showers of secondary electrons that amplify the radiation dose in the immediate vicinity of the NP, thereby enhancing nuclear DNA damage. However, later studies have shown that NPs do not penetrate the cell nucleus. Since most secondary electrons have only a very short range, this observation challenged this purely "physical hypothesis". Although some reactive oxygen species (ROS) generated by NPs have the potential to reach the cell nucleus, results on increased DNA damage in the presence of NPs remain contradictory. Thus, an alternative/additional target of NP-mediated radiosensitization could be cytoplasmic organelles, especially mitochondria possessing their own DNA. However, NPs do not colocalize spatially with mitochondria and the results regarding their damage in the presence of (irradiated) NPs are not clear. Therefore, increased damage to other organelles, such as lysosomes, in which NPs have been shown to accumulate, may also be considered.
In this presentation we will compare the effects of different types and sizes of irradiated and non-irradiated NPs on the cell nucleus (DNA fragmentation, chromatin architecture), cytoplasmic organelles (mitochondria and lysosomes) and the overall state of the cell as a system. Overall, our results to date suggest that NP-mediated radiosensitization is due to the integrative involvement of multiple mechanisms rather than a single mechanism that, together with the effects of radiation, stimulate the cell system to cell death.

References

- Dobešová L, et al. Incorporation of Low Concentrations of Gold Nanoparticles: Complex Effects on Radiation Response and Fate of Cancer Cells. Pharmaceutics 2022,14(1):166.
- Pagáčová E, et al. Challenges and Contradictions of Metal Nano-Particle Applications for Radio-Sensitivity Enhancement in Cancer Therapy. Int J Mol Sci. 2019 Jan 30;20(3).
- Stefancikova L, et al. Effect of Gadolinium-based nanoparticles on nuclear DNA damage and repair in glioblastoma tumor cells. J Nanobiotechnol. 2016;14(1):63.

Caterina Merla

C. Merla obtained MD and PhD in electronic engineering in 2004 and 2008 respectively from Sapienza University of Rome, Italy. Her main interests focus on biomedical applications of electromagnetic fields from an experimental and theoretical viewpoint. She is currently a researcher at ENEA, SSPT-TECS Division, Rome, Italy. She authored 60 papers on international peer review journals and hundreds of communications to international conference (also IEEE ones). She participated and leaded different national and international projects (e.g. H2020 Marie Sklodovska-Curie Individual Felloship MSC-IF, H2020 FET-OPEN grants). Dr Merla chaired and organized numerous sessions and special sessions on the topic to international conferences of the IEEE and served as council member of the European Bioelectromagnetic association from 2019 to 2021. She was also plenary speakers in various international conference in bioelectromagnetisms and optics. She is member of the IEEE and IMBioC 2020 committee. She is the general co-chair of the 5th World Congress on Electroporation held in Rome in September 2024.

Modulation of different biological processes by µs and ns pulsed electric fields

Pulsed electric fields of extremely short duration and high amplitudes represent an effective method for manipulating cells and tissues. These electric signals have the capability to alter the transmembrane potential of cell membranes, resulting in their rearrangement and the creation of hydrophilic nanopores. This phenomenon, known as electropermeabilization, can be harnessed for various biological and medical applications.
Our research group at the Laboratory of Biomedical Technologies of ENEA, has extensive experience in this field. We have utilized nanosecond and microsecond pulsed electric fields in oncological applications, employing both in vitro and in vivo models to destabilize cancer stem cells. This process induces their selective death through irreversible electroporation. Mechanisms related to the alteration of cancer stem cells have also been studied, identifying different pathways associated with the depletion of an important transmembrane protein, CD133, which is characteristic of cancer stem cells. This alteration consequently affects mitochondrial functionality, impairing cell viability. Additionally, we have investigated the effects of these signals in combination with ionizing radiation, both in vitro and in vivo.
Simultaneously, these signals have been employed to modulate the entry of specific molecules or ions, thereby altering cell functionality. A recent investigation focused on the modulation of calcium ion fluxes across the membranes of stem and cancer cells. Given the critical role of calcium in numerous cellular functions, this application holds promising potential for various medical treatments, ranging from regenerative medicine to cancer therapy.
During the workshop, we will present the methods and results related to these two research lines, also highlighting the future perspectives for our research.

Pietro Aprà

Pietro Aprà gained his MD in Physics in 2018 and PhD in 2022 at the University of Torino. His research activity has been focused mainly on diamond-based applications in biomedical field. More specifically, he worked on the tuning of nanodiamonds properties for applications in biolabeling and optical sensing, by means of ion beam techniques to create optically active lattice defects and by performing surface chemical modification processes aimed at making them suitable for biological applications. Currently he is a research fellow at National Institute for Nuclear Physics, holding the position of principal investigator of AURORA project, whose goal is the employment of nanodiamonds as simultaneous ROS quantum sensors and radiosensitizing agents.

Nanodiamonds for biomedicine: opportunities and challenges

During the last decades, nanobiotechnology turned out to be a promising research field, leading to the definition of novel nanosystems with application in biosensing, tissue engineering, drug delivery, nanodiagnostic and biolabeling. In this frame, nanodiamonds (NDs) are acquiring ever-increasing interest, due to their biocompatibility, chemical inertness, surface tunability and their fluorescence properties, arising from the presence of optically active lattice defects. One of the most interesting defects are the Nitrogen-Vacancy (NV) centers, which shows an intense red photoluminescence when excited with a green light source, thus providing a significant advantage for their use in optical bioimaging. In addition, the peculiar structure of the spin-dependent radiative transitions of the negatively charged NV centers allows for the optical detection of physico-chemical quantities such as weak electro-magnetic fields, small temperature variations within the biological samples under exams, by means of Optically Detected Magnetic Resonance (ODMR), thus disclosing a range of new perspectives in cell sensing with unprecedented spatial resolution and sensitivity. Moreover, optical relaxometry techniques can be employed to measure the presence of free radicals in the surrounding environment, providing wide perspectives in study of inflammation, aging, and radiobiology. To allow for these applications, one of the main challenges is the control and tuning of the surface and optical properties of NDs. To this scope, many techniques have been developed, focused especially on ion beam-based techniques to increase the NV centers amount, together with thermal and chemical modification processes to tune surface chemistry. Exemplary cases of surface functionalization with specific molecules and drugs aimed at tailoring the interaction with specific cancer cells will be shown, together with *in vitro* experiments designed to assess the actual potential of NDs in the biomedical context.

Alessandro Barge

Alessandro Barge got the degree in Chemistry in 1995 (University of Turin) and the PhD in Biochemical Sciences in 2000. In 2005 he was appointed as Assistant Professor (Organic Chemistry) at the University of Turin, Department of Pharmaceutical Sciences and Technology, and in 2017 he became Associate Professor (Organic Chemistry) at the same University and Department. His research started with the synthesis and characterisation of MRI contrast agents and, after 2005, it was implemented with the study of non-conventional techniques (such as US, MW and ionic liquids) applied to the organic synthesis and to the extraction of bioactive molecules from plant substrates. His research includes: organic synthesis of bioactive molecules, diagnostic systems, nanosystems (used in drug delivery, sensors, smart materials), as well as the green approach to synthetic processes. Among the different nanosystems, carbon-based nanomaterials cover a special role in his research: graphene, graphene reduced oxide, carbon nanotubes and nanodiamonds are some of the most studied nanomaterials. Organic synthesis is strongly supported by various analytical techniques:, NMR, mass spectrometry, GC, HPLC, IR. His teaching activities are dedicated to the courses "Physical Methods applied to Organic Chemistry" and "Organic Chemistry II" for the degree in Chemistry and Pharmaceutical Technologies. He is in charge of the NMR Open Access Laboratory of the University of Turin and has been consulted as an expert witness by the Public Prosecutor's Office. His research activity is evidenced by more than 105 publications in international journals and 5 patents.

Surface decoration of carbon-based nanomaterials for bio-medical applications

Carbon-based nanomaterials are proving to be particularly exciting for the development of biomedical nanosystems because of their special properties. Whether graphene, nanotubes or nanodiamonds, one of the key aspects of their use in such applications is the ability to modify their surface in a reproducible and possibly predictable manner. By incorporating appropriate molecular moieties on their surface, they can be further functionalised with a wide variety of molecules to create nanohybrids with unique properties. Surface functionalisation by pericyclic reactions or by the formation of amides has allowed the creation of nanosystems decorated with porphyrins, antibodies, hydroxyls and small organic molecules of biomedical interest, suitable for therapeutic, diagnostic or theragnostic purposes. In addition to the properties of the surface molecules, the nanodiamond has the typical properties of a carbonaceous material, characterised by a graphitic surface or, in the case of nanodiamonds, also by an sp3 core, which, depending on the defects present (e.g. NV centres), can exhibit fluorescence. The intrinsic fluorescence of the nanodiamond makes it a very interesting candidate for applications in diagnostics (targeted optical imaging), in smart surgery or in the development of in vitro diagnostic systems. Finally, the possibility of derivatising the nanosurfaces with different complementary reactive groups makes these nanohybrid structures versatile and widely exploitable for different applications. Some examples of this type of derivatisation are shown in this presentation.