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

19 April 2024
Aula Castagnoli - Dip. di Fisica
Via Pietro Giuria, 1 - Torino

For info: Federico Picollo → federico.picollo@unito.it, Veronica Varzi → veronica.varzi@unito.it

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

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
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 nanoparticles (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].

[2] E. Saracino et al., under review
[3] R. Fabbri et al., accepted for publication
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

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 Fellowship 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 electropereableization, 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.
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.
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 derivating 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.