Towards nanomedicine

Assets of hydrogenated detonation nanodiamonds for biomedical applications

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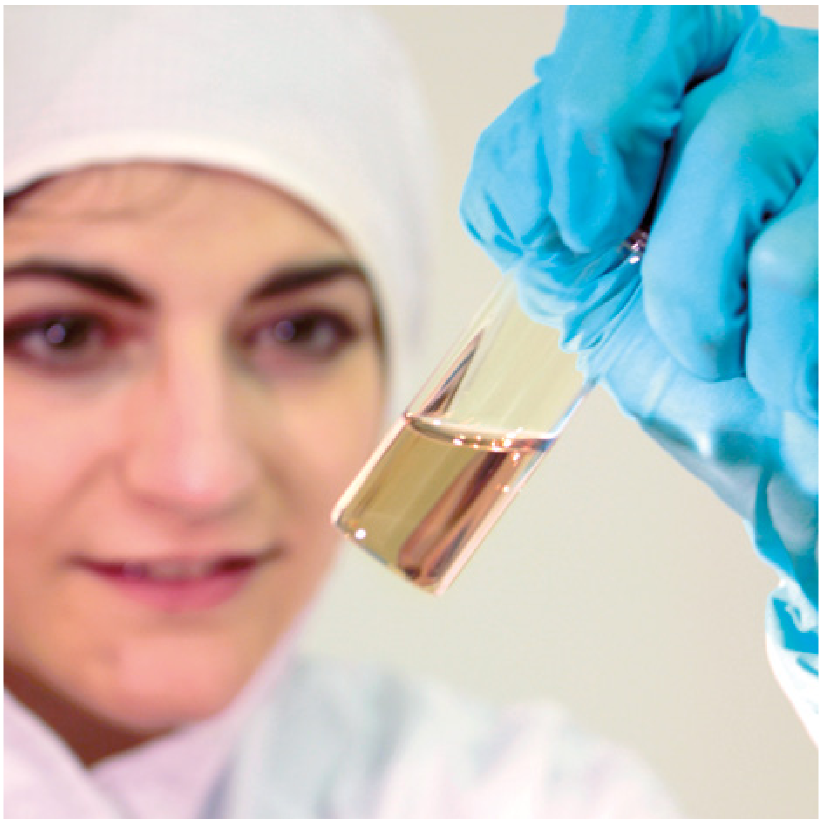
Diamond nanoparticles or nanodiamonds (NDs) behave several essential assets for biomedical applications: their very weak cytotoxicity and genotoxicity [1], their carbon-related surface chemistry for covalent functionalization of targeting or labeling moieties (oligonucleotides, proteins, fluorescent dyes, peptidic nucleic acids [2],…) and their tunable surface charge toward drug delivery [3]. In particular, detonation nanodiamonds synthetized by explosion [4] combine all this possibilities in a primary size of 5 nm, compatible with kidney filtration for an expected easier elimination. Nevertheless, these NDs are extremely sensitive to their surface state.



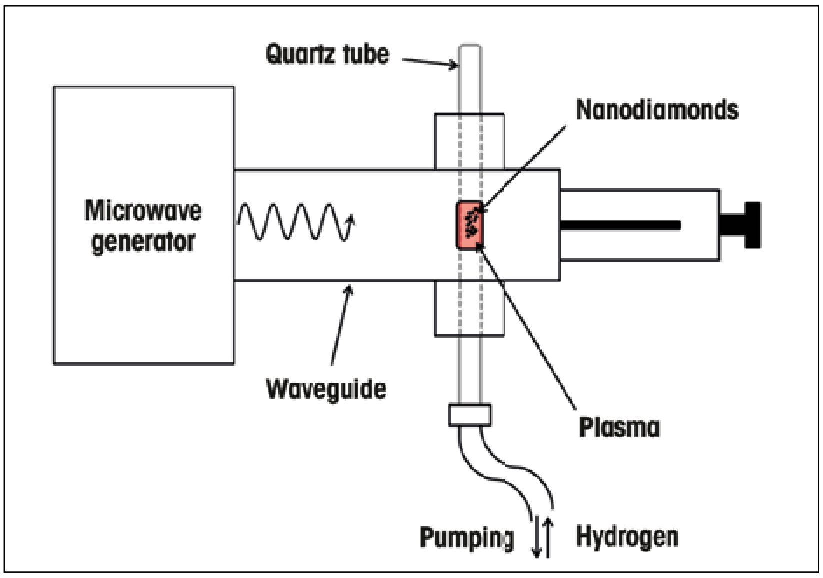


Fig. 1 Experimental set-up of hydrogenation

The Diamond Sensors Laboratory (CEA-LIST) has developed an expertise focused on controlled surface chemistry of NDs. Original treatments like plasma hydrogenation [5] or surface graphitization [6, 7] were optimized to get a homogeneous surface chemistry leading to specific electronic surface properties or functionalization routes. Such NDs possess a tunable surface charge and a very high colloidal stability in biological media.

High affinity for water molecules

Hydrogenated nanodiamonds were exposed to a microwave chemical vapor deposition plasma of hydrogen. 80 mg of nanodiamonds, precisely weighted on Mettler-Toledo Quantos (QB5-L), are treated each time. Atomic hydrogen produced under microwave field leads to the etching of non-diamond carbon, the reduction of oxygen species and the formation of C sp^3-H bonds. Kinetics of this hydrogenation technique were investigated by sequential surface analysis [8]. Using a home-made MPCVD reactor, optimal conditions were applied to produce large amounts of H-NDs (see fig. 1) [9]. Hydrogenated NDs (H-NDs) exhibit a diamond core surrounded by stable C-H1 terminations. A high affinity of H-NDs toward water molecules was demonstrated by adsorption isotherms (BET) with more hydrophilic sites compared to NDs-COOH [5]. As a consequence, stable H-NDs suspensions were obtained in water exhibiting a positive Zeta potential (ZP), + 45 mV at pH= 7.4 (see fig. 2). Its origin was related to a transfer doping occurring onto 5 nm diamond nanoparticles based on the diamond semi-conductive behavior.

The chemical reactivity of H-NDs was investigated via photochemical reaction with alkenes or diazonium chemistry. Their behaviour reveals similar to the one of hydrogenated diamond films [10].

F.l.t.r.: Dr Hugues Girard, Prof. Dr Jean-Charles Arnault, Dr Jacques de Sanoit, Céline Gesset

Hugues Girard received his PhD in Chemistry and Materials Science in 2008 at the University of Versailles, France. During his PhD, his research was focused on the electrochemical reactivity of diamond electrodes. From 2008 to 2010, he took a postdoctoral position between the French Atomic Energy Commission (CEA) and Ecole Polytechnique, where he gained expertise on the surface chemistry of diamond nanoparticles. In 2010, he joined Diamond Sensors Laboratory (CEA) as a full time researcher, where his interests relate mainly to the biological and technological applications of diamond nanoparticles. His expertise led to the development of original methods for nanodiamond plasma treatment, aiming at their surface hydrogenation and more recently their radioactive labelling.

Jean-Charles Arnault is a research director at the atomic energy and alternative energies commission (CEA). After obtaining his PhD degree in 1993, he was an assistant professor at Strasbourg University until 2007, when he joined the Diamond Sensors Laboratory at CEA. His prior expertise concerns diamond nucleation and growth, interactions between MPCVD plasmas with surfaces using in situ surface analysis methods and electron microscopies. Since 2008, his research has focused on diamond nanoparticles. He initiated a new research field at CEA dealing with surface modifications of diamond nanoparticles using plasma or thermal treatments to control their surface terminations and confer new surface properties. Recently, hydrogenated nanodiamonds revealed solid assets for bioapplications in particular a radiation sensitisation effect.

Jacques de Sanoit has been a research scientist at CEA and holds 35 years of experience. After 10 years of research and development in the field of nuclear fuel reprocessing and the radiochemical separation of the minor actinides, he spent 15 years at the Henri Becquerel National Laboratory (CEA-LIST) where he was in charge of the preparation of the radioactive sources for primary metrology of ionizing radiations. For 10 years, he joined the Diamond Sensors Laboratory (CEA-LIST) where he conducts research on electrochemical sensors.

Céline Gesset graduated from Télécom SudParis in 2004 with a specialty in optics and high frequency sensors. She joined CEA in 2005 to developed new design of sensor. Since 2008, she is a staff member at the Diamond Sensors Laboratory, where her main research topic is based on the study of nanodiamonds for sensors and biological applications. Her principal activity is the understanding of surface modifications of nanodiamond particles due to different treatments (chemical, gas, etc..), their characterizations and the measure, in different media, of their colloidal properties.

Fig. 2 Colloidal suspension of hydrogenated nanodiamonds dispersed in water (3 mg/mL)

Efficent against tumour cells

Hydrogen terminations can also confer new electronic properties to NDs, which can be promoted toward radiosensitization effect for cancer treatment. Indeed, an in vitro radiosensitisation effect for hydrogenated 5 nm NDs was demonstrated on radioresistant cell lines under gamma irradiation (collaboration Experimental Cancerology Laboratory; CEA iRCM) [11]. Under X-rays irradiation, H-NDs are able to generate a high concentration of reactive oxygen species (ROS) which could be efficiently used against tumoral cells. The ROS production might be in relation with the specific electronic properties of hydrogenated surface i.e. its negative electron affinity and strong reactivity toward water molecules [5]. Biological effects are under investigations [12].

Nanoparticle radiotracers are currently widely used to assess quantitatively the health hazard related to nanotechnologies or as theranostic agents [13]. Replacing the hydrogen gas by tritium during the plasma, this surface chemistry can be easily turned to C-H^3 thus providing intrinsic radiotracing properties to the particles, essential prerequisite to a biodistribution study. A radioactive labeling of the diamond core itself appears as a promising approach that ensures the tracing of NDs. Such a direct radioactive labeling using plasma treatment has been recently achieved [14]. The total radioactivity measured by liquid scintillation includes 93 % of H^3 atoms tightly bonded to the NDs surface and 7 % embedded into the diamond core. Such H^3 doping ensure highly stable radiolabeled nanodiamonds on which surface functionalization is still allowed.

Summary

Carrying a positive zeta potential desirable for delivery and offering a diamond core with therapeutic potentialities, H-NDs have now reached a maturity for nanomedicine. Nanodiamonds were previously considered as an efficient but inert platform. We demonstrated that NDs and more specifically H-NDs should now be considered as an active nanomaterial.

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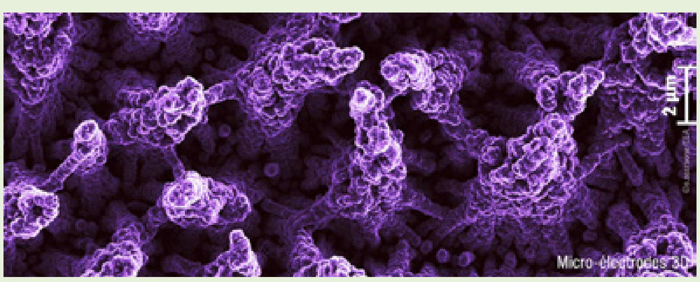
Research activities of Diamond Sensors Laboratory are focused on CVD diamond growth and its innovative applications. According to its outstanding properties, diamond can address very different industrial challenges: sensors for hostile environment, innovative sensors, functional coatings, bio-interfaces, …

Diamond films are grown for protective coatings, tribology or biology applications and high-quality substrates for sensors and detectors design. Our research topics also concern physical and chemical properties of diamond films and nanodiamonds surfaces (functionalisation, plasma/surface interaction, bio-interfaces, …)

Our technological platforms, our scientific expertise and our numerous collaborations allow to develop applications in very different areas: security, health, nanotechnology, synchrotron facilities, etc.

■ **http://cnanoidf.org/fr/recherche/nanochimie-nc/equipes/equipes-paris-sud-orsay-palaiseau/article/laboratoire-capteurs-diamant-cea**



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