Nanomedicine: concrete achievements thanks to interdisciplinarity

Abstract Nanomedicine can be defined as the medicine involving nanomaterials and nanotechnologies for theranostic approaches. The possibility of integrating particles of nanometric dimensions in pharmaceutical formulations has transformed the medical world, finding applications in various fields. So far, nanoparticles enable to make innovative breakthroughs in the development of nanovaccines, the targeted delivery of drugs, nano-antibiotics as alternatives to combat bacterial resistance and infections, as well as the development of new and miniaturized diagnostic platforms, and the design of medical implants and wearable devices for personalized medicine. Some of these innovations are currently used in clinics. Some of our contributions to this multidisciplinary field at the interface between chemistry, materials science, nanotechnology, biology and medicine are presented in this article.

Keywords **Nanomedicine, graphene, cardiovascular biomarkers, nanovaccines, nano-antibiotics.**

Résumé **La nanomédecine : des réalisations concrètes grâce à l'interdisciplinarité**
La nanomédecine peut être définie comme la médecine impliquant des nanomatériaux et des nanotechnologies dans les approches théranostiques. La possibilité d'intégrer des particules de dimensions nanométriques applicables chez l'homme dans des formulations pharmaceutiques a bouleversé le monde médical, en trouvant des applications dans divers domaines. Les nanoparticules interviennent aujourd'hui dans de nombreuses avancées innovantes, notamment le développement de nanovaccins, l'administration ciblée de médicaments, les nano-antibiotiques pour lutter contre la résistance bactérienne et les infections bactériennes, mais aussi dans le développement de plateformes de diagnostics miniaturisées, ainsi que la conception d'implants médicaux et de dispositifs portables pour la médecine personnalisée. Cet article présente quelques contributions des auteurs dans ce domaine pluri- et interdisciplinaire où se croisent chimie, science des matériaux, nanotechnologie, biologie et médecine.

Mots-clés **Nanomédecine, graphène, biomarqueurs cardiovasculaires, nanovaccins, nano-antibiotiques.**

Nanomedicine has rapidly progressed from dream to reality over the last years. This is a fascinating interdisciplinary field, where nanotechnology meets life sciences and clinic. The name appeared in the 1990s and since then it has experienced an exponential increase of interest in the scientific community, notably in the last decade and probably pushed by the statement of the US government to fund nanomedical studies [1]. At the European level, EuroNanoMed, a platform established in 2008 to support multidisciplinary, translational research and innovation projects that cover regenerative medicine, diagnostics and targeted delivery systems, supports actively the nanomedicine research community [2]. The field of nanomedicine provides a completely new set of tools for researchers and in particular for clinicians with the underlying goal to discover better devices for early diagnosis or treatment of a variety of diseases with the overall objective being the enhancement of patient's quality of life. Nanomedicine also enables to optimize and/or develop innovative delivery methods of medicines in a highly efficient and specific manner, thereby improving compliance and reducing side-effect risks of medicines. With this regard, nanomedicine is pivotal for personalized medicine. So far, nanomedicine covers a much broader range of research and development comprising countless novel nanotherapeutics, nanodiagnostics and nanotheranostics technologies that expand our ability to manage, monitor, predict and eventually prevent diseases (*figure 1*).
Our group is contributing to this broad field of nanomedicine *via* the bias of materials science and nanotechnology often

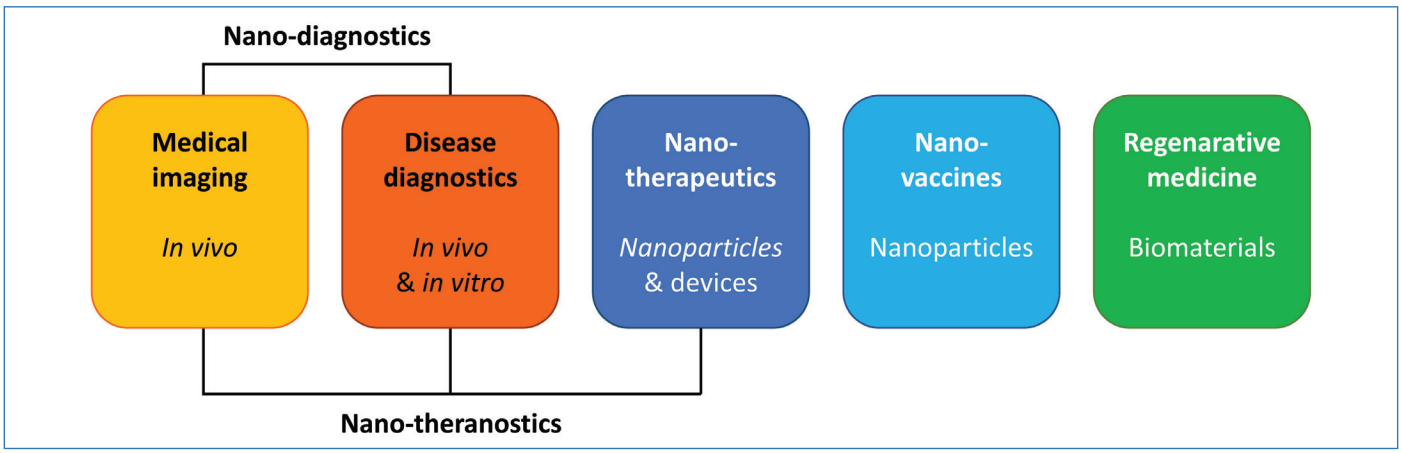

Figure 1 - Different areas of nanomedicine.

connected with the establishing of adapted *in vitro* and more lately *in vivo* assays such as:
- ***disease diagnosis*** in biological fluids using electrochemical and plasmonic sensors for selective and sensitive detection of biomarkers, notably those related to cardiovascular diseases; fast and selective detection of microorganisms such as uropathogenic *E. coli*, responsible for hospital acquired and community-acquired urinary tract infections (UTIs);
- ***nanotherapeutics*** through the development of photo-thermal active materials and interfaces in the near-infrared allowing photothermal ablation of pathogens, enhanced transdermal drug delivery as well as the local treatment of skin infections;
- treatment of viral infections using carbon quantum dots (CQDs) up to the testing of nano-adjuvants for ***nanovaccine formulations***.

Nanodiagnostics

Electrochemical platform for sensing of cardiac biomarkers in serum and saliva

Cardiovascular diseases remain one of the leading causes of death within industrialized nations. Despite the great efforts put into the development of biochemical-based diagnostic approaches, these techniques remain medical challenges. Our group has contributed to this field through the development of cardiovascular disease biomarker sensors working in serum as well as in saliva [3-5]. Measuring biomarkers level in saliva rather than in serum samples opens the possibility for non-invasive, painless sensing, something of high importance for elderly and fragile patients. The reason while only a limited number of saliva-based sensors are reported is correlated to the largely decreased concentration level of most biomarkers (below picomolar) when compared to that in serum. The use of nitrogen-doped porous reduced graphene oxide (N-prGO) modified electrodes with a cardiac troponin I (cTnI) specific aptamer allowed detecting cTnI down to 1 pg mL^{-1} in serum and saliva (*figure 2*) [5]. Not only is the sensor well adapted for clinical settings and allows the differentiation between healthy and acute myocardial injury (AMI) diagnosed patients, it also postulated a saliva cTnI level of 675 pg mL^{-1} in AMI diagnosed patients. Encouraged by these preliminary results, clinical studies on saliva samples from healthy and AMI diagnosed patients are ongoing at the CHU Lille with the aim to answer questions related to the pharmacokinetics of cardiac biomarkers in saliva and to allow the development of a portable point-of-care device in the close future.

Selective isolation and sensing of uropathogenic *E. coli*

Urinary tract infections (UTIs) are one of the most common bacterial infectious diseases in humans, with *Escherichia coli* being the most predominant pathogen responsible for hospital and community-acquired UTIs. As pathogenic *E. coli* bacteria are able to infect and cause disease to the host tissues, it is of high importance to detect these microorganisms at a very low level and be able to discriminate between different bacterial strains. Bacteria culturing techniques are commonly used for bacteria detection [6]. However, these methods take a full day to rule out a negative sample, and the analysis may require up to several days to confirm a positive result, making them unsuitable for on-site monitoring. In this context, the possibility to selectively isolate *E. coli* associated with urinary tract infection from serum samples using functionalized magnetic nanoparticles and their detection is an important step further [7]. An optimized magnetic nanocarrier (*figure 3*), composed of nitrodopamine-coated magnetic particles (MP_{ND}) embedded onto PEG modified reduced graphene oxide (rGO-PEG) nanosheets, to which *E. coli* specific anti-fimbrial antibodies were covalently linked, displayed several important features:
- 99.9% capture efficiency even at *E. coli* UTI89 concentrations of only 10 cfu mL^{-1} in 30 min;
- specific elimination of *E. coli* UTI89 from serum samples;
total photothermal ablation of *E. coli* UTI89*;*
- functionalization with fluorescently-labelled anti-*E. coli* anti-bodies allowed *E. coli* UTI89 sensing down to 10 cfu mL^{-1}.

Nanotherapeutics: the interest of reduced oxide nanomaterials

Advances in materials science have largely contributed to the refinements of current drug delivery systems. Along with the ability to improve drug target specificity, nanotherapeutics

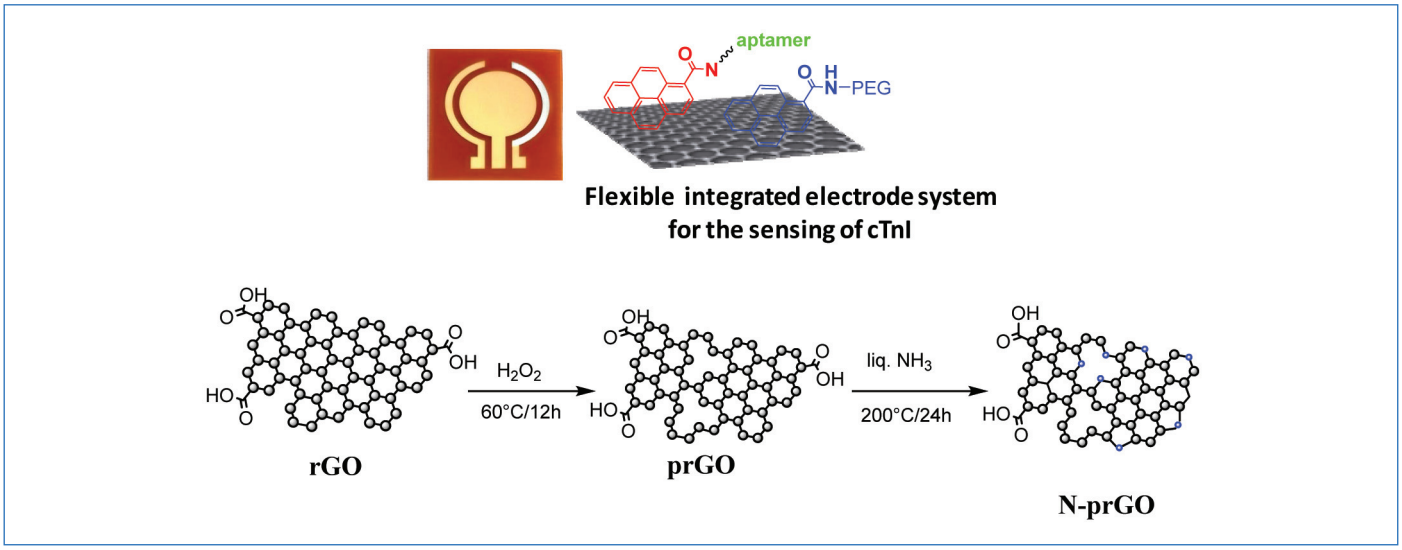

Figure 2 - Electrochemical cardiovascular sensor working in serum and saliva: fabrication process of cTnI sensor based on nitrogen-doped porous reduced graphene oxide (N-prGO) modified with pyrene-poly(ethylene glycol) (pyrene-PEG) as antifouling unit and pyrene-aptamers for cTnI specificity (reprint with permission from [5]).

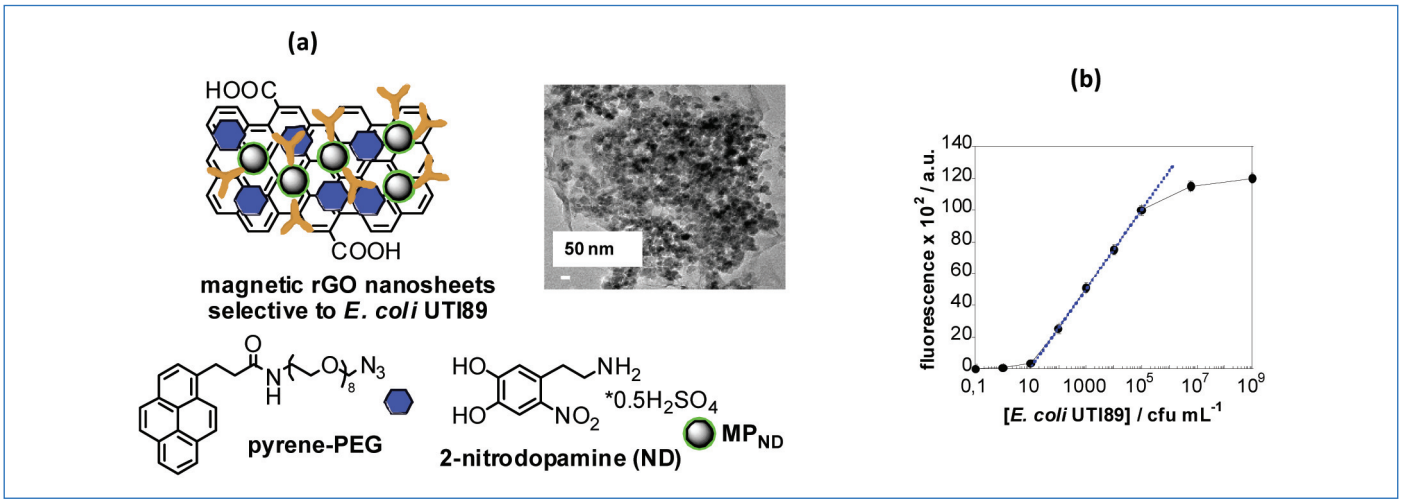

Figure 3 - Selective isolation and sensing of *E. coli* UTI89 with magnetic reduced graphene: (a) schematics of materials and molecules used for the construction of the isolation and sensing matrix (inset: TEM image of matrix) (reprint with permission from [7]); (b) calibration curve for *E. coli* UTI89 (unpublished results).

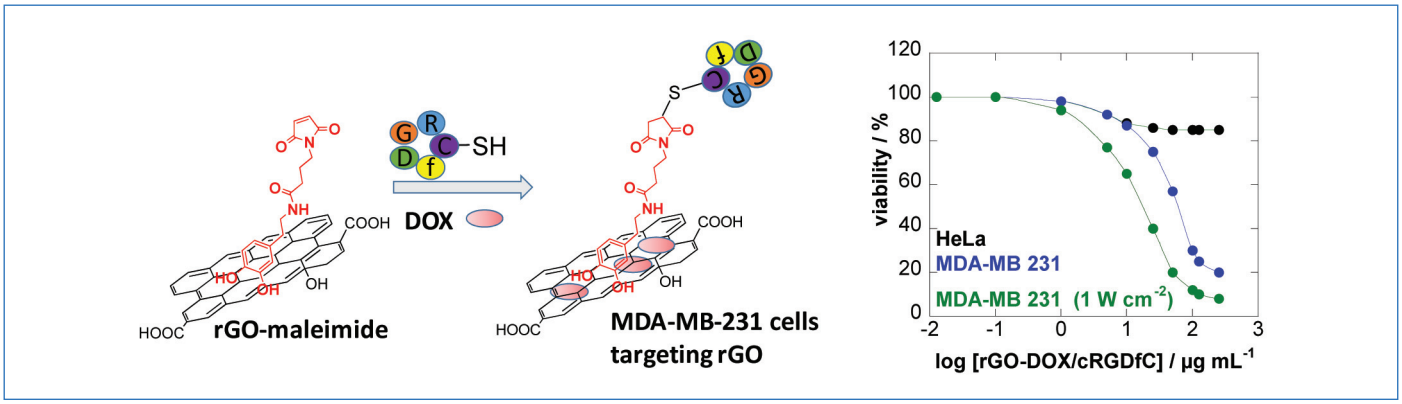

Figure 4 - Targeted cancer cells ablation using DOX loaded c(RGDfC) modified reduced graphene oxide nanosheets. Formation of targeting matrix together with dose response curves (reprint with permission from [11]).

allow the use of reduced drug volumes, avoiding the problem of accumulation in healthy tissue. The developments of stimuli-responsive nanotherapeutics, inducing a therapeutic effect in response to an externally controlled stimulus, promise unique clinical benefits over conventional systems that release their cargo passively [8-10]. Controlled drug release in response to illumination at a specific wavelength has the advantage of being non-invasive with the possibility of remote spatio-temporal heating when using photothermal active materials. Such an approach allowed for the selective ablation of MDA-MB-231 by taking advantage of the efficient light-to-heat conversion of reduced graphene oxide (rGO) nanosheets [11-14], the high loading capacity of rGO for anticancer drugs such as doxorubicin (DOX) and the possibility to further integrate cancer cell targeting peptides (*figure 4*) [11].

While rGO formulations are ultimately to be used in the form of intravenous injections, graphene-based matrixes have also proved to be ideally suited for increasing the permeability of therapeutics *via* the skin in a concept named photothermal-assisted transdermal drug delivery [13-15]. Most of the proposed transdermal drug delivery (TDD) systems are based on passive diffusion through the skin, a process that is suitable for effective delivery of macromolecular therapeutics such as insulin and other proteins [16]. A number of different innovative approaches have been explored over the years to align TDD with the life sciences [15]. The interest of heat [15] for the delivery of small [13] as well as macromolecular drugs [14] was lately demonstrated by us. Taking the example of transdermal insulin delivery (*figure 5* p. 26), the 3D structure of PEG-based rGO hydrogels is favourable for the integration of insulin, while the strong light absorption of the rGO-hydrogel in the near-infrared (NIR) results in controlled release of insulin at therapeutically relevant concentrations in a few minutes. Most importantly, the photothermal-induced insulin release has no bearing on its biological activity and has been able to regulate the blood glucose level in mice.

The photothermal effect of rGO is further extremely efficient for the treatment of skin infections [17-20]. Dermal injuries render the human body significantly vulnerable to infections and the formation of subcutaneous bacterial abscesses. Current treatment approaches involve incision-induced wound drainage and administration of high doses of antibiotics intravenously, as topical applications often exhibit limited or no healing effect. The importance of temperature in the wound-healing process and as alternative to antibiotic treatment for the effective killing of pathogens has been recognized as a novel way to treat wound infections (*figure 6* p. 26). The temperature rises by several tens of degrees upon light activation of the rGO based topical heating dressing, adequate for the treatment of infected skin at an early stage [18]. The reusability of the patch, together with the possibility to sterilize it, recommends this method as cost-effective and potentially marketable approach.

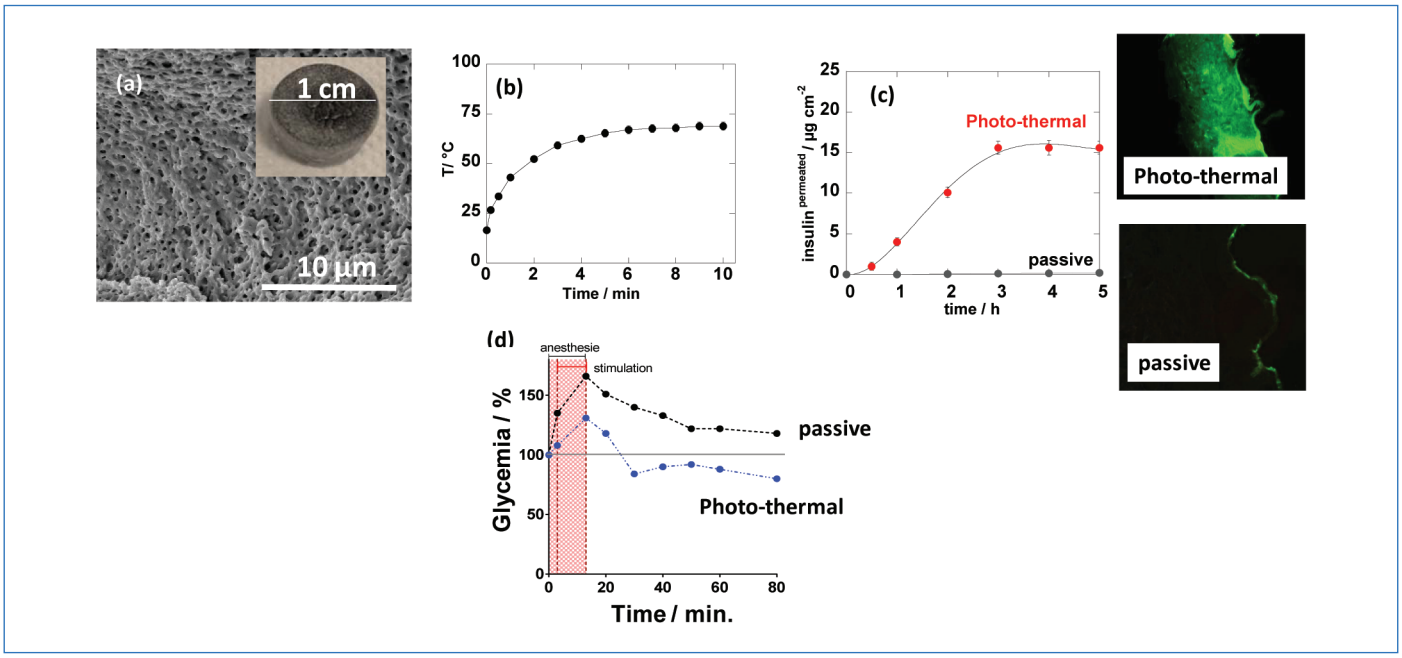

Figure 5 - Photothermal-enhanced transdermal insulin delivery: (a) SEM and photographic image of an insulin loaded rGO-hydrogel; (b) change in solution temperature during illumination of insulin loaded rGO-hydrogels; (c) insulin permeation profiles through porcin skin together with fluorescence sections; (d) change of blood glucose level of mice with application of insulin loaded rGO-hydrogel for 10 min without (black) and with light activation (blue).

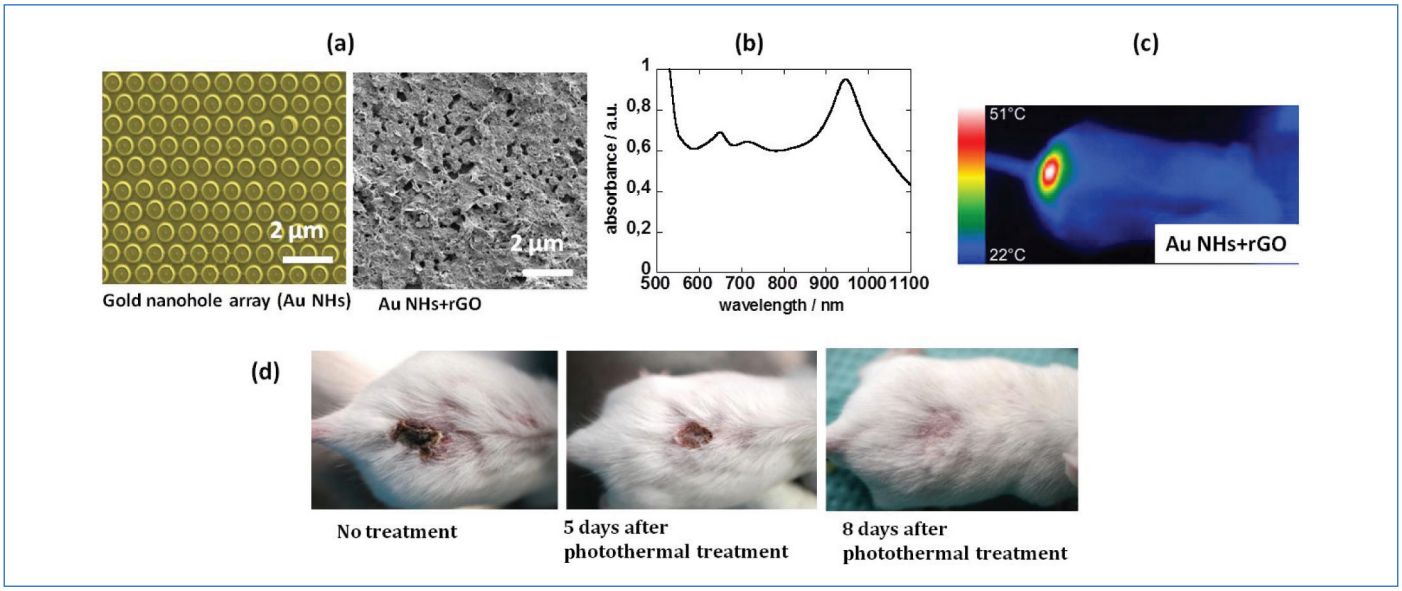

Figure 6 - Topical heating dressings for the treatment of subcutaneous skin infections: (a) SEM images of gold nanoholes (Au NHs) and Au NHs post-coated with rGO; (b) typical absorption spectrum of the topical dressing; (c) thermal images of mice treated with the flexible Au NHs/rGO nanoheater; (d) photographs of mice after five days of infection without treatment and with photothermal treatment (reprint with permission from [18]).

Nanovaccines: from the treatment of viral infections using CQDs to the use of nano-adjuvants for vaccine formulations

The eradication of viral infections is an ongoing challenge in the medical field, not only due to the problem of spreading but also to virus' ability to escape therapy by genetic mutations. The lack of targeted antiviral therapeutics as well as the constant emergence of new viruses make the search for antiviral agents a challenging and extremely needed research task. Several nanoscale materials have been proposed by us as alternative to effectively modulate the viral infection cycle [21-23]. More recently, a carbon quantum dots (CQDs) based strategy for the treatment of human coronavirus infections was proposed (*figure 7*) [24]. Human coronaviruses (HCoVs) are one of the World Health Organization (WHO) listed emerging pathogens with great epidemic potential. While circulating HCoVs cause relatively mild common cold-like respiratory tract infections, the Middle-East respiratory syndrome coronavirus (MERS-CoV) leads to pneumonia requiring hospitalization and intensive care. A total of 2266 laboratory-confirmed cases of MERS-CoV, including 804 associated deaths, have been declared to WHO until now, with a high case-fatality rate (35%). As the virus is circulating in animals and humans, it may undergo further adaptation and causes a pandemic. Screening a large variety CQDs for their possibility of treatment of

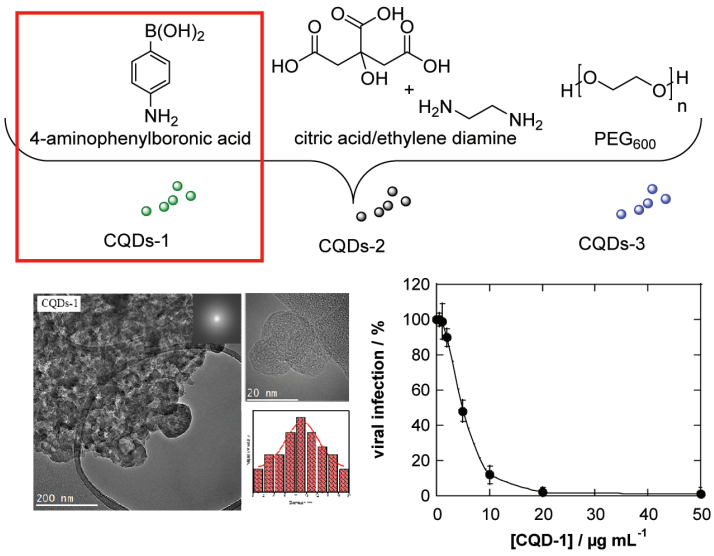

Figure 7 - Treatment of human coronavirus infections using functional carbon quantum dots: schematic representation of the hydrothermal carbonization of different organic precursors for the synthesis of CQDs together with TEM image and viral inhibition activity of CQDs-1 formed from 4-aminophenylboronic acid (reprint with permission from [24]).

human coronavirus infections allowed the identification of one effective CQD, CQDs-1, currently proposed as the first-generation anti-HCoV nanomaterial.

Closely linked to the development of antiviral strategies is the development of vaccines against these viruses. Many immunogens used in vaccine formulations require the use of adjuvants, substances which enhance the immune response to the specific antigens present in the vaccine against a given disease [25]. While this research field is currently under development in our group within a maturation project ("Actions d'initiatives régionales pour la recherche (AIRR)" Volet Start-AIRR, Nanodiamond based vaccine against community-acquired infections), the idea of using nanomaterials in vaccine formulations was lately materialized by testing aluminium oxide nanowires (Al_2O_3 NWs) kindly provided by Prof. Yushin's group at the Georgia Institute of Technology (USA), as a safer and effective adjuvant for next-generation vaccines (*figure 8*) [26]. When injecting Al_2O_3 NWs of 20-60 µm length and 20-40 nm in diameter into mice in the presence of ovalbumin (OVA) as model antigen, a four times stronger humoral immune response as compared to alum (a common adjuvant) was observed. In parallel, Prof. Bilyy's group at Danylo Halytsky Lviv National Medical University (Lviv, Ukraine) could validate that these nanostructures are boosting significantly the activation of white blood cells known as neutrophils without any damaging effects on the blood capillaries and microvasculature. The set of different adjuvant properties of ultra-long Al_2O_3 NWs holds great promise for their rapid implementation as a safer and a more effective adjuvant alternative for human vaccines as they permit to achieving similar adjuvant effects at lower concentrations. Moreover, it is expected that modifying the surface chemistry of the nanowires, currently under development in our group, and optimizing their dimensions and morphology may boost their performance further [27].

Synergy will be the key

Over the years, a large portion of the nanotechnology promises for the medical field has been achieved. The challenges and future trends in nanodiagnostics are to bringing the different diagnostic devices from hospital settings to the home of chronically ill patients in the form of point-of-care (POC) devices. This requires the integration of sensors onto stretchable/compressible bandages. As these devices should provide day-to-day diagnosis at home or when abroad and promote immediate decision, making wireless data transmission is needed. While this thematic is outside of the competences of our team, they are all present at the Institute IEMN and will allow to be treated adequately. What our team aims at, however, is the integration of self-regulated drug-delivery systems upon a health-alarming sensor signal. The best-known example is glucose-dependent insulin delivery with however large difficulties associated with making an implantable closed system that has both glucose sensing

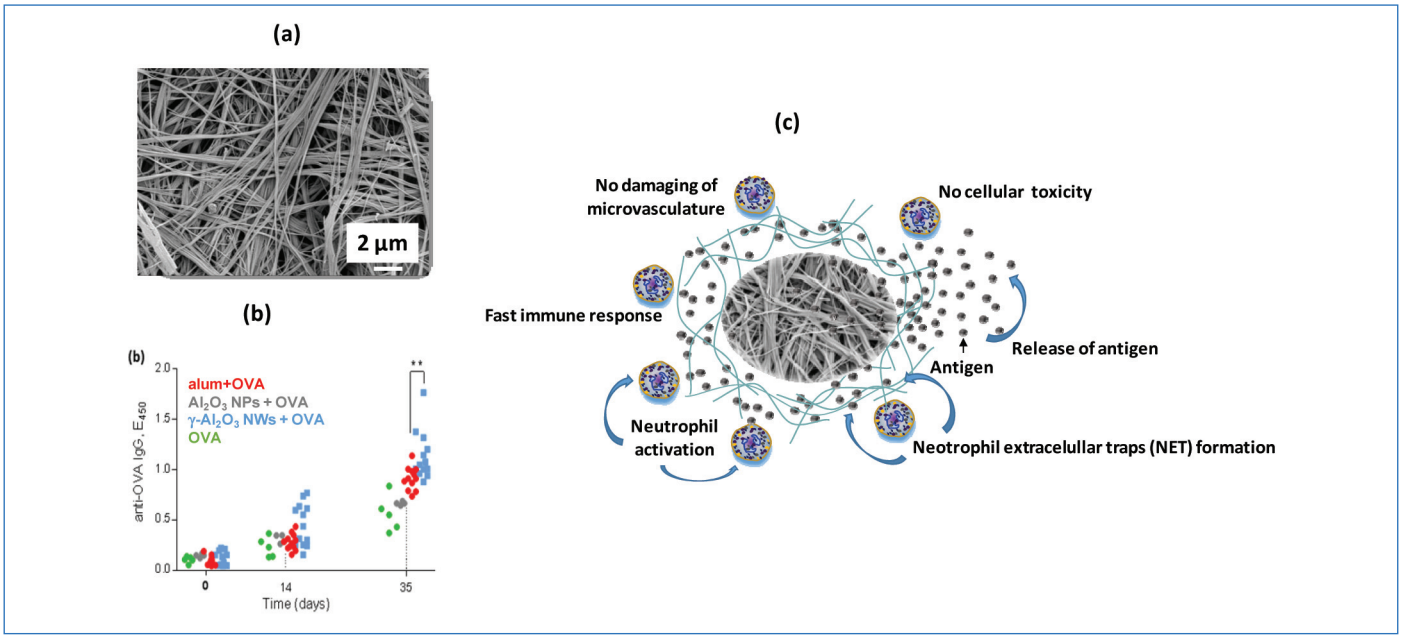

Figure 8 - Ultra-long γ-Al_2O_3 nanowires as next generation adjuvants: (a) SEM micrograph of γ-Al_2O_3 nanowires; (b) humoral immune response in mice, (c) advantages of ultra-long Al_2O_3 NWs (reprint with permission from [26]).

and insulin release capabilities. However, a combination of a skin patch taking advantage of photothermal triggered transdermal drug delivery and the possibility to measure cardiac biomarkers in sweat is a possible future way to overcome some of the technological hurdle of implanted sensors.

Heat-based therapeutic systems are attained surprisingly easily and at low cost, when taking advantage of photothermal active agents such as reduced graphene oxide and others. The perspectives of this approach are countless. One of our current interests is the application of photothermal heating bandages to treated diabetic foot ulcers. Indeed, if glycemia is not well managed or not treated, diabetes can progress into several complications including foot ulcers, chronic wounds and difficult to treat. Smart heating bandages are expected to increase locally the low oxygen content. Together with a local release of painkillers and/or antibiotics, the ability of these wound ulcers to become non-healing might be reduced with a large positive effect on patients. Turning the potential of nanotechnology into clinically useful formulations and outcomes requires however a close interplay between materials scientists, biologists and medical doctors to set up clear and realistic goals. The future of nanomedicine is not only linked to the implementation of new ideas and concepts, but to finding a real synergy between nanotechnologists, materials scientists, biologists and clinicians, open for testing the initial clinical proof of concept of new nanomedicines and devices.

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