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THESE DE DOCTORAT EN COTUTELLE

PRESENTEE DEVANT L'UNIVERSITE DE LILLE 1

- *VU la loi n° 84-52 du 26 janvier 1984 et notamment son article 17*
- *VU le décret n° 84-573 du 5 juillet 1984*
- *VU l'arrêté du 7 août 2006 relatif aux études doctorales*
- *VU les rapports de*

GRANDJEAN Cyril - Chargé de Recherche CNRS - Université de Nantes
ZAPOROZHET Olga - Professeur - Kiev National University

LE PRESIDENT DE L'UNIVERSITE DE LILLE 1 :
1) Autorise Mr TURCHENIUK VOLODYMYR
à soutenir une thèse de Doctorat en :

SCIENCES DES MATERIAUX

sur le sujet suivant :
FUNCTIONALISATION OF NANOPARTICLES AND
THEIR BIOMEDICAL APPLICATIONS

2) Désigne :
DIRECTEUR - SZUNERITS Sabine - Professeur des Universités - Université Lille 1
CO-DIRECTEUR - ZAITSEV Vladimir - Professeur - Taras Shevchenko National University
RAPPORTEUR - GRANDJEAN Cyril - Chargé de Recherche CNRS - Université de Nantes
RAPPORTEUR - ZAPOROZHET Olga - Professeur - Kiev National University
INVITE - TANANAIKO Oksana - Professeur - Kiev National University

pour constituer le jury de soutenance de cette thèse.

3) La soutenance aura lieu le 03-10-2016 à 09 Heures Kiev National University, Kieve, Ukraine

Villeneuve d'Ascq le 27/09/2016

UNIVERSITE LILLE1 SCIENCES ET ... DIRVED ETUDES DOCTORALES

Pour le Président
Le Vice-Président
Recherche
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Nantes, August 25th 2016

Rapport sur la thèse présentée par Monsieur Volodymyr TURCHENIUK en vue d'obtenir le grade de Docteur de l'Université de Lille 1 / Review of the PhD thesis presented by Mr Volodymyr TURCHENIUK to obtain the rank of Doctor from the University of Lille

Mr Volodymyr TURCHENIUK presents a PhD thesis entitled *"Functionalization and modification of nanoparticles and their biomedical application"*, describing the work he carried out under the co-supervision of Professors Sabine SZUNERITS and Vladimir ZAITSEV at the "Institut d'Electronique, de Microélectronique et de Nanotechnologie (IEMN-UMR CNRS 8520)" at the Université de Lille and at the Kiev National University (Ukraine), respectively.

The work reported by Mr Volodymyr TURCHENIUK deals with the design, the synthesis and the characterization of novel nanoparticles for the treatment of bacterial infections or cancers. Research on nanoparticles has recently emerged as one of the most active fields of research. Indeed, it has been shown that nanoparticles could have a deep impact on, among others, materials, energy, environment or biomedical applications. It is therefore important to note that the work realized by Mr TURCHENIUK took place in a very innovative and internationally competitive area.

The manuscript, divided into seven Chapters, is preceded by an introduction highlighting the main objectives of the work.
The first Chapter corresponds to a bibliographic section which gives an overview on nanoparticles for biological applications. Mr TURCHENIUK has made a brief statement on the specific properties of the nanostructures which render them attractive for such applications as well as a listing of both organic and inorganic materials which have been used so far. In the remaining part of Chapter 1, he has focused on gold nanoparticles and carbon nanodiamonds which form the two main structures which have been investigated during his thesis. Of note, a particular emphasis on the photothermal and photodynamic properties of the former is provided.

The scientific results obtained by Mr TURCHENIUK are described in the following five Chapters. He has decided to adopt a form similar (but not identical) to the so-called "thesis of publications" to present his data. As a consequence, each Chapter is independent from the others and comprises an introduction, a result & discussion section, a conclusion and a separate bibliography. On the one hand, one might consider that such organization harms the unity of the thesis, the pros and cons of the different approaches not really compared between them while some redundancies cannot be avoided. On the other hand, the adopted strategy has the merit of clarity, giving rise to brief Chapters, focused on one biological issue and providing a solution thanks to the design of novel hybrid nanostructures.

Chapter II describes the preparation of carbon nanodiamonds decorated with different sugars to interfere specifically with lectins (non enzyme proteins which bind to sugars) expressed at the surface of bacterial pathogens. The main originality of the work relies on the strategy developed to attach the sugars onto the carbon nanodiamond surfaces. The strategy implies the photoactivation of arylated azide groups linked to the carbon nanodiamonds to afford nitrenes which, in turn, react with free sugars in absence of any kind of activation.

Mr TURCHENIUK has continued his quest of novel antibacterial agents in Chapter III. He thus planned to combine the known, moderate antibacterial activity of menthol with that of carbon nanodiamond particles. The resulting conjugated nanoparticles did not display bactericidal activity but were able to inhibit biofilm formation for two major pathogens, *Staphylococcus aureus* and *Escherichia coli*, a feature barely met by other types of nanoparticles.

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In Chapter IV, he switched from carbon nanodiamonds to gold nanorod/SiO_2 core-shell nanostructures in which a phorphyrin derivative - verteporfin - was embedded. Both verteporfin and gold nanorods possess interesting photodynamic properties. Their combination showed synergistic effects leading to novel photosensitizers which showed promises for antibacterial near infrared two-photon photodynamic therapy using either continuous or pulse laser sources.

The preparation of novel gold nanorods/reduced graphene oxide core – shell nanocomposites as well as their assessment in photo-thermal cancer therapy is reported in Chapter V. Photo-thermal therapy is a novel minimally-invasive therapy which relies on laser irradiation of a structure (likewise plasmonic gold nanorods) able to convert photon energy into heat. In turn, the local increase of temperature which is induced leads to the death of surrounding cells. Compared to the state of the art in the field, the main objective of the project consisted in limiting the laser energy required to observe the photo-thermal effect so as to prevent side-effects such as burning of safe tissues. The designed nanostructures proved to be effective using a laser power of 0.5-0.7 W cm^{-2} below that of 1 W cm^{-2} usually applied. The nanostructures decorated with Tat peptides as a targeting device and Cy7 molecules as fluorescent probes have been tested *in vitro* and *in vivo*. A tropism for glyoblastoma cells was observed for these nanocomposites whilst they showed effective photo-thermal therapy in a mouse model of glioblastoma.

The last Chapter of results is related to Chapter V. Mr TURCHENIUK has reported the first *in vitro* photo-thermal treatment of cancer cells using nanocomposites whereby gold and palladium are combined with reduced graphene oxide nanosheets.

The manuscript ends up with a brief conclusion & perspectives chapter (Chaper VII) and an experimental part.
All nanostructures described in the PhD thesis have been consistently characterized using a set of complementary techniques such as Fourier transformed infrared spectroscopy, micro Raman spectroscopy, X-ray photon spectroscopy as well as transmission or scanning electron microscopy... This certifies that Mr TURCHENIUK has accomplished a huge work at the bench and acquired many skills that will be useful to him in his future career. The manuscript will certainly gain in clarity if the English writing and the overall layout (noticeably the places where the figures are inserted) were improved (separate comments will be sent to Mr TURCHENIUK by the reviewer along this line). Moreover, this successful work is obviously the result of a fruitful multi-disciplinary partnership involving chemists covering synthetic organic and inorganic chemistry as well as physical-chemistry, experts in nanotechnology, biochemists and biologists. Whereas Mr TURCHENIUK's manuscript demonstrates that he can master and account for experiments carried out by collaborators, the exact contribution of each partner is not clearly stated within the manuscript.

The outstanding work described by Mr TURCHENIUK, though not yet fully published, has already led to seven publications in high impact, both specialized and general scope journals such as *Chem. Commun.* or *J. Mater. Chem. B*. Mr TURCHENIUK appears in first or second position on the list of authors in four out of these seven publications.

The reviewer thus expresses an **opinion very favorable to the thesis defense of Mr Volodymyr TERCHENIUK.** Le rapporteur donne donc un **avis très favorable à la soutenance de thèse de Monsieur Volodymyr TERCHENIUK.**

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Doctorante: VOLODYMYR TURCHENIUK
Title de la These: Functionalization and modification of nanoparticles and their biomedical application
Universite de Lille 1 Ecole Doctorale Regionale Sciences pour l'Ingenieur Lille Nord-de-France

The work performed in the frame of the doctoral thesis be dials the synthesis, modification and characterizations of number nanoparticles on the base nanodimonds, silica-encased gold nanorods, bimetallic AuPd NPs on rGO nanosheets for using in nanomedicine.

In Chapter I, the candidate presents a review. He demonstrates that the integrating biological components with nanomaterials can revolutionize the field of pharmacology and help to tackle diseases at a molecular level. This chapter is clear written. Briefly describes well known nanodiamonds, gold nanorods that proposed for antibacterial application and are analysed current approaches to the use of nanoparticles of different nature in nanomedicine. This chapter is a good introduction to the field in particular for non-specialists and it helps to highlight the innovations described by the candidate in the experimental part. But the review is a too short, it based on 48 references. In the thesis, different surface modification strategies have been developed. An effort was put mainly on the surface modification of nanodiamonds (NDs), gold NPs (Au-NPs), Au-Pd bimetallic nanocomposites. At the same time, a critical analysis of modification strategies of nanoparticles of different nature in In Chapter 1 is absent.

In Chapter II, the candidate proposed a new method of nanodiamond modification with different lectins. In literature there only few publications with methods that gives us some possibility to put sugars on nanodiamond surface. The main methods are click-chemistry reaction based on formation of triazole ring with different propargyl modified sugars. But such synthesis are not easy to performed. In this work a photochemical linking of unmodified mono-, di-, and polysaccharides to nanodiamond particles pre-modified with perfluorophenylazide ligands was proposed as a general method for coupling underivatized carbohydrates to diamond nanostructures. The diamonds prepared in this way demonstrated a high inhibitory efficiency of the ND-mannose and other glycan-modified particles and nanostructures was against E. coli.
But if the selection of modifying agents justified, the choice of the method of their immobilization including the synthesis of azido-modified nanodimonds is not good argumented . Moreover in the thesis are no data on the effect of the nature of "spencer" on the activity and toxicity of nanoparticles.
Unfortunately, in the work the hydrolytic stability of the modified nanoparticles, its resistance to washing in a buffer medium is not discussed. In this and another cases, the biological activity of the modified nanoparticles should be compared with the activity of a mixture containing unmodified nanoparticle (including "spencer") and free modifying agent.

Chapter III was aimed at the demonstration of the menthol-conjugated nanodiamond effects on S. aureus and E. coli-mediated biofilm formation. The antibacterial properties of menthol are well know. The results obtained in the work prove that nanodimond can be modified with acid derivative of menthol without losing activity. It was showed that ND-menthol particles are capable of effective prevention of bacterial adhesion of Gram-negative and Gram-positive bacteria.

Chapter IV, was base on another route of nanoparticles – gold nanorods. In this work I should mark that the crucial point was in development of synthesis of gold nanorods as it not easy despite a lot of work exist and based on this synthesis. But another thing was to make this particle to increase possibility of photosensitizer Verteporphyn in producing of reactive single oxygen species. So as we see the photodynamic properties of this particles show us a great effect. This method may be considered as one of the alternate strategies for destroying of wound-infected antibiotic resistants.

Previous Chapter based on photodynamic properties of gold nanorods, but as we see in **Chapter V** they also could be used as particles for photothermal effect. In this work was investigated that Au NRs, which is covered with pegylated rGO (Au NRs@rGO-PEG), are perfect multi-functional therapeutic nanostructures, which are able to exert effective photothermal deactivation of tumors in mice on NIR light excitation and are able to operate as fluorescent markers of cells. Big deal was made in work with mice and important to say that this multioperational theranostic

nanostructures ran effective photothermal elimination of tumors in mice by low dosage of NIR light excitation.

In Chapter VI was showed a green and elementary synthetic method for the creation of bimetallic AuPd NPs on rGO nanosheets, that stabilized with PEG and have a good solubility in water. This particles showed nice photothermal effect owing to synergetic effect. Important to say that PEG was attached covalently. This research may reveal new opportunities of development of improved materials for using as potential photothermal variants for therapy of various unregulated cells.
But the description of methods for determination of the contents of gold and palladium (in varying degrees) in the phase of nanoparticles is absent.
Unfortunately, the effect of the synthesis conditions (pH, concentration of ascorbic acid, modifying agent, etc.) and the nature and concentration of immobilized metal on the nanocomposite properties is not discussed.In..

The Chaper VII briefs conclusion & perspectives chapter) and an experimental part.

For nanoparticle characterization were used a number complementary techniques such as transmission and scanning electron microscopy, X-ray photon-, IR- and micro Raman spectroscopy. This work is the result of a fruitful multi-disciplinary partnership in the field of synthetic organic and inorganic chemistry, physical-chemistry, nanotechnology, biochemists and medicine. The scientific results are published in seven journals with high impact factor.

In the thesis were proposed: the azido-modified nanodimonds for photochemical reaction with sugars, the menthol modified nanodiamond particles as bacterial viability against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria, novel nanostructures for photodynamic therapy comprising silica-encased gold nanorods modified with verteporfin as photosensitizer and the efficiency of these nanostructures for killing of pathogens, bimetallic AuPd NPs on rGO nanosheets for photothermal therapy. But the results would be more convincing if the author has compared the efficiency of proposed nanoparticles and the known nanoparticles of a different nature.

The reviewer thus expresses an **opinion very favorable to the thesis defense of Mr. Volodymyr TURCHENIUK.**

Olga Zaporozhets