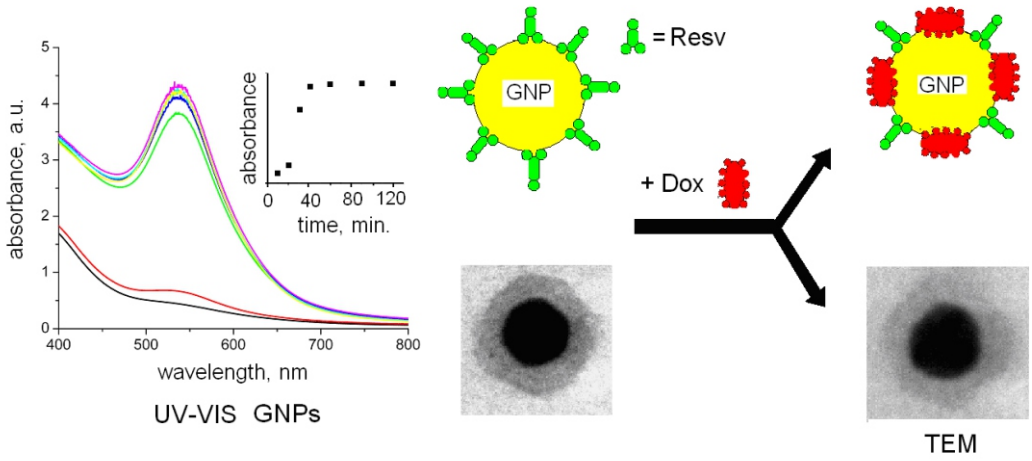
Maria Tomoaia-Cotisel

MULTIFUNCTIONAL NANOSTRUCTURES FORMED OF GOLD OR SILVER NANOPARTICLES AND DIFFERENT BIOMOLECULES WITH MEDICAL APPLICATIONS



Presa Universitară Clujeană / Cluj University Press

MULTIFUNCTIONAL NANOSTRUCTURES FORMED OF GOLD OR SILVER NANOPARTICLES AND DIFFERENT BIOMOLECULES WITH MEDICAL APPLICATIONS

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PRESA UNIVERSITARĂ CLUJEANĂ / CLUJ UNIVERSITY PRESS

2016

Acknowledgements. **We are grateful for support from the UEFISCDI through the NANOMED project of exploratory research (type PCE) and grant no. 257.**

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ISBN 978-606-37-0017-0

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51 Hasdeu Str.
400371 Cluj-Napoca, Romania
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Contents

Preface

This monography presents an overview of our recent developments [1-41], mainly from the laboratories of Physical Chemistry Center, Department of Chemical Engineering, Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University of Cluj-Napoca, in the field of multifunctional nanostructures formed of gold or silver nanoparticles and different biomolecules along with their potential medical applications in nanomedicine. In addition, collaborative research was done in various other laboratories of authors engaged in this research as partners, and are indicated in the original research articles and patents, cited as reference.

The major objective of our work was the development of innovative strategies for nanostructure processing and characterization of biomolecules [1-12], in bulk of aqueous solutions and at the interfaces. Another important objective was the development of novel synthesis strategies to obtain the controlled narrow size distribution of gold nanoparticles, GNPs, [13-26] and of silver nanoparticles, AgNPs, [15, 27-30]. Then, an important objective was the coating of GNP surface [16-26] or of AgNP surface [27-29] with biomolecules by adsorption and self-assembly. Thus, the functionalization of GNP surface with synthetic or natural organic molecules, such as amino acids (e.g., arginine, cysteine, lysine), anesthetics, chitosan and globular protein as well as tannin, collagen, resveratrol and doxorubicin, improved their stability by preventing their aggregation, under suitable reaction parameters [16-26]. Similar results were obtained by functionalization of AgNP surface with beta-cyclodextrin, alpha-lipoic acid, amino acids or globular protein [27-29].

Certainly, the interaction between the obtained multifunctional nanostructures and biological systems depends on the properties of constituent nanoparticles, namely size, shape, crystallinity, porosity, surface charge, surface chemical functionality and their chemical composition, and was thoroughly investigated in two human cervical tumor cell lines [26] and on microbial pathogens [30, 31] or in healthy human osteoblast cultures [32, 33].

Introduction

Nanostructures of biomolecules

For the beginning, we display our original research articles, which describe the strategies developed by us for nanostructure processing and characterization of biomolecules [1-12], exploring their self-assemblies in bulk and at interfaces [1-9] as well as their effect on biological membrane, for example on human erythrocyte membrane [10], or on model membranes, for instance Langmuir-Blodgett (LB) lipid membranes [11, 12].

The biomolecules, such as globular storage protein extracted from aleurone cells of barley, were thoroughly physical, chemical and biochemical investigated [1-5] and further used in premiere to functionalize gold nanoparticles [20-22] possessing high potential for the development of innovative drug delivery systems.

Among biomolecules, collagen type 1 was also used and its supramolecular organization with anti-cancer drugs, e.g., 5-fluorouracyl and doxorubicin, was investigated [6, 7]. The obtained results open a new opportunity for further research and applications of collagen fibers as carriers of anti-cancer drugs in vitro and in vivo.

Another important research was conducted using anti-oxidants, like alpha-lipoic acid and beta-cyclodextrin, and their nanostructures were chemically and physically characterized [8, 9]. They were further used for the production of gold and silver nanoparticles [15, 27] with potential applications as adjuvants in cancer therapy.

Furthermore, we present our results on manufacturing and nanostructure processing and on characterization of developed nanostructures, which are incorporated in original research articles, cited as references [13-35], and in patent [36]. They are based on gold and silver nanoparticles, functionalized with various biomolecules, and on ceramic nanoparticles, forming a multifunctional platform for biomedical applications using nanoscale methods and advanced nanotechnology.

Nanotechnology deals with particles of size less than 100 nm in their diameter, and especially involves the manipulation of individual atoms and molecules. It is used in

the development of nanostructured materials, and involves the production and the nanostructure processing that are decisive for the properties and the characteristics of the resulting nanomaterials as well as for their applications.

Nanostructures of gold nanoparticles

Our advanced nanotechnology can control particle size, shape and surface properties of various inorganic nanoparticles. For instance, GNPs are synthesized using the reduction of $HAuCl_4$ by citrate, where the citrate acts both as the reducing agent and the stabilizer [13]. The size of the GNPs can be controlled by varying the ratio of reaction precursors. We also applied methods of green chemistry for the synthesis of GNPs via plant extracts [14], or by using beta-cyclodextrin [15], or resveratrol [26].

Specifically, GNPs were further used to investigate their interaction with various biomolecules, such as amino acids: lysine [16], arginine [17], cysteine [18, 19], leading to the formation of GNPs - amino acid complexes and self-assemblies with high selectivity for each amino acid. These results support the potential application of GNPs for the purpose of sensing amino acids in vitro. In consequence, GNPs can be used as sensors to determine various amino acids from different biological media. The said GNPs - amino acid complexes are also promising tools for in vivo therapeutic purpose, such as drug delivery into cancer cells [26]. Undoubtedly, due to the nano size, these tools are suitable for direct interaction with various cellular subunits and are of great further research interest.

Another well-designed method for the safe production of GNPs utilizes an improved Turkevich method and functionalization of the GNP surface with globular protein molecules. This synthetic strategy [20, 21] was developed and reported by us, for the first time, in the state of the art. The multitude of binding sites on the protein layer adsorbed on GNPs offers many opportunities for further selective modification of GNP surface. This aspect brings important benefit for drug delivery and for their internalization into the cells.

The new concept of utilizing nanoparticles of globular protein, such as the protein extracted from aleurone cells of barley [1-5], as a template for the creation of novel nanostructures of GNPs was introduced by us almost one decade ago [20, 21]. This novel idea is based on our previous innovative fundamental concepts incorporated in patents [37-40] and in research article [41], designed for drug delivery using peptides through the blood brain barrier to the brain.

Further GNPs were functionalized with amino acids and aleurone globular protein molecules [22] as well as with chitosan and aleurone globular protein [23, 24], resulting important nanomaterials with controlled optical properties. The GNPs were also studied in the interaction with local anesthetics: procaine, tetracaine and dibucaine [25] and a selective effect was determined, showing that GNPs can be used in the detection process of various anesthetics in biological media.

The green synthesis of GNPs and the modification of the GNP surface with biomolecules, such as trans-resveratrol, Res, have provided an efficient transport of GNP-Res complexes into the cervical cancer cells, e.g. HeLa and CaSki cells, with minimal cytotoxicity, for the low concentrations used by us. The GNP-Res complexes were further functionalized with doxorubicin, Dox, and the resulted GNP-Res-Dox complexes had a substantial cytotoxic effect on the two cervical cancer cells [26], even at low and safe dose of Dox, usually recommended for in vivo administration. These nano complexes demonstrated an efficient delivery of Dox to the cancer cells, representing our premier discovery in the field of cervical cancer. Also, it is rational to disclose that these complexes escaped endosomes ensuring a specific Dox targeting to the cellular nuclei without an additional functionalization with extra moieties, such as cell penetrating peptides, known to be useful for cellular internalization. Moreover, the apoptosis induction in HeLa and CaSki cells was strongly evidenced for GNPs-Res-Dox complexes by flow cytometry, leading to cellular mitochondrial dysfunction. This remarkable effect will be explored in future studies at different concentrations in complexes and also on various types of cancers. Furthermore, as evidenced by MTT cell viability assay, GNPs-Res-Dox complexes inhibit proliferation of HeLa and CaSki cells in dose dependent of GNPs and these studies will be also advanced in future studies.

Additionally, our preliminary data indicate no cytotoxicity of GNPs in bone marrow mesenchymal stem cell (BMSCs) culture opening a new assumption that GNPs are promising particles to be added to hydroxyapatite in a controlled manner for bone regeneration. We intend to further investigate their effect on osteogenic differentiation of human stem cells. In this context, it appears reasonable to emphasize an open remark, on our discoveries related to a possible major breakthrough in nanomaterials, particularly in multifunctional nano hydroxyapatites enriched in gold nanoparticles, which might enhance osteogenic differentiation of human stem cells.

Nanostructures of silver nanoparticles

Furthermore, AgNPs were generated by reduction of silver nitrate in the presence of alpha-lipoic acid, which is a compound covering a key role in cellular metabolism, having remarkable antioxidant properties [27]. The AgNPs were also obtained by green synthesis [15] by using beta-cyclodextrin. Both alpha-lipoic acid and beta-cyclodextrin had a remarkable stabilizing effect on AgNPs dispersions.

The investigation of self-assembled arrangements of AgNPs in the presence of amino acids and protein [28] disclosed a strong functionalization of AgNPs with potential applicability in biomedical research. Finally, a new procedure was developed to synthesize AgNPs of controlled size by using glucose in alkaline media [29]. These AgNPs showed a strong interaction with local anesthetics: procaine, tetracaine and dibucaine. Even more, the selective interaction of AgNPs and anesthetics was also determined [29], which can be exploited to evaluate the concentration of anesthetics in various media.

The AgNPs, obtained by reduction of silver nitrate via glucose procedure [29], were also used to determine their antimicrobial effect in composites made with multi-substituted hydroxyapatite, containing low concentrations in Ag, Au, and Zn, embedded in a polymer matrix realized with bis-GMA and TEG-DMA [30]. A strong antimicrobial effect was determined and correlated with the Ag^+ ions release profile for various concentrations of AgNPs in said composites [30]. The antimicrobial effect was also studied on Ag^+ ions along with the antibiotic nitroxoline, adsorbed on hydroxyapatite disks [31]. Pure hydroxyapatite and triple-substituted hydroxyapatite, used in systems with AgNPs, were obtained by wet precipitation method [30-36].

Methods used for nanostructures characterization

The globular storage protein extracted from aleurone cells of barley was investigated by different physico-chemical methods: FTIR, NMR, Raman spectroscopy, fluorescence spectroscopy, in order to clarify its structure, as well as by TEM and AFM to identify its nanostructures realized by its adsorption from aqueous dispersions on solid surface [1-5].

The supramolecular organization and the formation of collagen nanostructures with anti-cancer drugs were also studied by AFM and STM [6,7]. The structure of the inclusion complex of α-lipoic acid with β-cyclodextrin was elucidated by XRD, DSC,

FTIR, Raman and NMR [8,9]. The interaction of anesthetics and human erythrocyte membranes was explored by AFM, cross section profiles in 2D AFM images as well as by roughness measurements [10].

The effect of procaine on lipid domains, realized in model membranes, such as Langmuir spread monolayers at water/air interfaces, was determined by using compression isotherms, e.g. lateral surface pressure versus mean molecular area. At a constant surface pressure the mixed procaine-lipid layers were transferred, by Langmuir-Blodgett (LB) technique, on glass support and visualized by AFM imaging in contact mode [11]. Results showed a strong effect of anesthetics on lipid membranes and a substantial expanding effect was recorded and correlated with interfacial phenomena occurring during anesthesia. The phase transition from liquid expanded to liquid condensed in phospholipid monolayers was also studied by AFM and LB technique and rheological properties were evaluated [12].

GNPs or AgNPs in the absence or the presence of various biomolecules in aqueous dispersions were investigated by UV-Vis spectroscopy, AFM and TEM, Also, on solid GNPs or AgNPs powders, FTIR, Raman and XRD were used to explore the characteristics of GNPs and AgNPs [13-29].

The antimicrobial inhibitory effect of AgNPs and of Ag^+ ions was determined by Kirby-Bauer technique [30, 31]. The Ag^+ ions release profile was determined [30] by inductively coupled plasma-optical emission spectrometry (ICP-OES). Hydroxyapatite nanopowders were investigated by XRD, TG, DTA, FTIR, Raman, TEM, SEM, AFM, BET analysis and zeta potential measurements [30-36].

This monography shows our results on the interactions of the developed nano-structured materials with different biological systems, in vitro, using two lines of cervical cancer (HeLa and CaSki) [26] as well as against different microbial pathogens [30, 31] or in human healthy osteoblast cultures [32, 33]. Through the efforts of innovative development, an optimal composition of these nanomaterials might be determined, and in consequence, our strategies might hold potential applications for cancer therapy, antimicrobial coatings and engineered bone scaffolds. Finally, this monography provides a perspective on the effects of these obtained results. The future research in this outlined field will bring new possible challenges for advanced nanomaterials with medical applicability in nanomedicine research.

Chapter 1: Biomolecules

Chapter 2: Gold Nanoparticles

Chapter 3: Gold Nanoparticles and Cervical Cancer

Chapter 4: Silver Nanoparticles

Chapter 5: Silver Nanoparticles, Silver Ions and Antimicrobial Effect

Chapter 6: Hydroxyapatite Nanoparticles

Perspective and concluding remarks

This monography shows an important evidence that biomolecules, such as globular and fibrous proteins, amino acids, lipids, carbohydrates, anti-oxidant compounds and therapeutic agents, can be used in nanostructures [1-12] to functionalize and in consequence, to increase the stability of inorganic nanoparticles, like metallic nanoparticles or ceramic nanoparticles. They can also bring the biomolecular signalling when the resulting nanostructured biomaterials are used as scaffolds or can convey advantages of molecular recognition when are used as tools in drug delivery investigations in cell culture or in animals.

Metallic nanoparticles can be extensively used in various applications due to their nano size and extensive thermal stability [13-30]. Among these particles, gold nanoparticles appear to be preferred in therapeutic applications because they absorb strongly the light and can generate thermal energy which induces photothermal destruction of malignant tissue.

Particularly, gold nanoparticles are a clear choice for therapeutic applications due to their easy synthesis and functionalization, and because are less toxic in vivo than AgNPs and ease of detection. Certainly, functionalization facilitates for targeted delivery of these nanoparticles to various cell types, lead to drug delivery and tissue imaging, and in consequence, they possess various therapeutic and diagnostic applications.

Regarding the GNPs applications, a fundamental question appears related to the possible toxic effect of GNPs on human healthy cells. Actually, in the state of knowledge many studies on GNPs cytotoxicity are inconsistent so far. Depending on the size of gold particles as well as on the biomolecules adsorbed on them, the binding strength of biomolecules to the gold surface and the surface functionalities on their outermost layer adsorbed on GNPs might play a critical role in the cellular uptake and in possible intracellular changes and thus, on their cytotoxicity.

Nowadays, it is not possible to give a general statement about the toxicity of GNPs, because more systematic studies are needed. Important research effort is necessary

to define the different parameters, which are playing a critical role in the complex interaction between nanoparticles and cells.

Our results from this monography cover several broad categories of GNPs functionalized with organic molecules, such as amino acids, like lysine, arginine and cysteine [16-19], globular storage protein extracted from aleurone cells of barley [20-22], chitosan and globular protein [23, 24], anesthetics [25], as well as antioxidants (resveratrol) and anti-cancer drugs (doxorubicin) [26] by chemical adsorption or by self-assembly. Consequently, there is convincing evidence for a potential application of GNPs to cancer therapy but further development is required prior to cancer clinical trials.

The AgNPs are also functionalized with beta-cyclodextrin [15], alpha-lipoic acid [27], amino acids and globular protein [28], anesthetics [29], and show high thermal stability and strong antibacterial effects under suitable conditions.

Another generation of nanostructured composites are developed and made of ceramic nanoparticles, primarily nano hydroxyapatite: HAP and AgNPs, embedded into polymeric matrix [30]. They show a strong antimicrobial effect against several different pathogenic species: *Escherichia coli, Staphylococcus aureus*, *Staphylococcus spp., Bacillus cereus,* and *Candida albicans,* using the Kirby-Bauer disk-diffusion method [30]. These composites can be considered as promising antimicrobial materials for coating of orthopedic and dental implants or used as bone cements in surgical applications.

The antimicrobial effect of ceramic disks loaded with silver ions in the presence of an antibiotic: nitroxoline (5-nitro, 8-hydroxy quinoline: NHQ) is also determined against *Staphylococcus aureus* [31]. Results demonstrate, for the first time in state of the art, the ability of NHQ and of Ag^+ to exert their antimicrobial effect by using HAP disks. This technique offers another approach for delivering antimicrobial agents, such as NHQ and silver ions, within ceramics disks for their local delivery with potential applications to prevent or treat infections. Further studies are needed to evaluate the efficacy of NHQ and silver ions impregnated HAP disks against microbial pathogens. This investigation can be extended to various pathogens to address the susceptibility of microbial biofilms to NHQ and Ag^+ ions, incorporated in ceramic disks. The nanostructure processing of ceramics, such as calcium nano-phosphates and pure hydroxyapatite (HAP) or multi-substituted HAP, used in formulations with AgNPs, is shown in articles [30-35]. The synthesis of HAP, with controlled size of particles, is realized by wet precipitation method [36].

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ISBN: 978-606-37-0017-0