Applied Surface Science 437 (2018) 110–121

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Full Length Article

Nanocomposites based on graphene oxide and mesoporous silica nanoparticles: Preparation, characterization and nanobiointeractions with red blood cells and human plasma proteins

Leandro C. Fonseca[a,*], Maciel M. de Araújo[a], Ana Carolina M. de Moraes[a], Douglas S. da Silva[a], Ariane G. Ferreira[b], Lidiane S. Franqui[b,c], Diego Stéfani T. Martinez[b,c], Oswaldo L. Alves[a,*]

[a] Laboratory of Solid State Chemistry, Institute of Chemistry, Universidade Estadual de Campinas, 13083-970, Campinas, São Paulo, Brazil
[b] Brazilian Nanotechnology National Laboratory (LNNano), Brazilian Center for Research in Energy and Materials (CNPEM), 13083-970, Campinas, São Paulo, Brazil
[c] School of Technology, Universidade Estadual de Campinas, 13484-332, Limeira, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 15 August 2017
Received in revised form 5 December 2017
Accepted 9 December 2017
Available online 23 December 2017

Keywords:
Amine nucleophile
EDC
NHS
1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
N-Hydroxysuccinimide
Crosslinking agents

ABSTRACT

The current work refers to the development of a novel nanocomposite based on graphene oxide (GO) and mesoporous amino silica nanoparticles (H_2N-MSNs) and its biological interaction with red blood cells (RBCs) and human blood plasma toward the investigation of nanobiointeractions. Silica nanoparticles and several graphene oxide-based materials are, separately, known for their high hemolytic potential and strong interaction with human plasma proteins. In this context, the GO-MSN interaction and its influence in minimizing the reported effects were investigated. The materials were synthesized by covalently attaching H_2N-MSNs onto the surface of GO through an amidation reaction. GO-MSN nanocomposites were obtained by varying the mass of H_2N-MSNs and were characterized by FTIR, NMR, XRD, TGA, zeta potential and TEM. The characterization results confirm that nanocomposites were obtained, suggest covalent bond attachment mostly by amine-epoxy reactions and evidence an unexpected reduction reaction of GO by H_2N-MSNs, whose mechanism is proposed. Biological assays showed a decrease of hemolysis (RBC lysis) and a minimization of the interaction with human plasma proteins (protein corona formation). These are important findings toward achieving *in vivo* biocompatibility and understanding the nanobiointeractions. Finally, this work opens possibilities for new nanomedicine applications of GO-MSN nanocomposites, such as drug delivery system.

1. Introduction

The interaction of nanostructures with biological systems is one of the crucial scientific investigations to understand the effects of molecular carriers as drug delivery systems on living organisms in which the intravenous medium is one of the main routes of application. Considering human blood as the means of circulating the nanomaterial, it is necessary to analyze the way in which the nanocarrier interacts with the present cellular systems, comprising red blood cells (also called erythrocytes or RBCs), white blood cells (also named leukocytes or WBCs) and platelets (or thrombocytes) [1–3]. In addition, it is also important to understand nanomaterial-protein interactions (protein corona formation) in order to predict biochemical and physicochemical phenomena regarding the toxicity of the material. The interaction of nanostructures with blood proteins corresponds to the area of nanomedicine, which investigates, among numerous possibilities, the relation of the effect of protein adsorption on the surface of nanomaterials and the further reaction of the immune system, as foreign bodies of the blood (alluding to the nanocarriers) can be eliminated from the bloodstream through their interaction with immune system proteins called opsonins, forming a nanocarrier-opsonin complex. The nanocarrier is recognized by macrophages (a type of leukocyte) and subsequently phagocytosed by said white cell in a mechanism called opsonization, decreasing the blood circulation time of the nanocarrier and, thus, decreasing the probability of reaching the target cell [4,5]. Thus, studies related to the interaction between

* Corresponding authors.
E-mail addresses: leandro.fonseca89@gmail.com (L.C. Fonseca), oalves@iqm.unicamp.br (O.L. Alves).

https://doi.org/10.1016/j.apsusc.2017.12.082
0169-4332/

nanomaterials and proteins provide important information for the comprehension of possible biochemical reactions and biological responses generated after their application in the blood and can serve as a mimetization of the opsonization mechanisms [5–7].

Among the studied materials at the interface of chemistry and biology, graphene oxide (GO) is the subject of study in several scientific and technological works. GO is a two-dimensional carbon material comprising a region of carbon atoms with sp^2 hybridization (similar to the intrinsic structure of pristine graphene) [8] and another where sp^3 carbons are associated with oxygenated functions, according to the most accepted structural model described by LerfKlinowski [9]. GO has functional epoxy and hydroxyl groups located in its basal plane and carbonyl and carboxylic acid groups situated preferentially at the edges of the two-dimensional sheets, providing it with the possibility of several functionalizations in the process of the acquisition of new physicochemical properties of interest [9]. This material intrinsically has interesting properties, such as high hydrophilicity, the ability to form dispersions in water and several organic solvents, high mechanical strength, significant theoretical surface area ($2630\ m^2\ g^{-1}$), and optical transparency [10,11]. These important properties of GO contribute for its wide possibilities of applications as photocondutive switching [12], bioimaging [13], photocatalysis [14], biosensors [15], supercapacitors [16] and for the recent progress in water desalination [17]. However, its application and/or interaction with biological systems is still a new area, and in this scenario, graphene oxide is a potential candidate for such applications due to its biocompatibility and low toxicity when functionalized with key molecules or hybrid nanoparticles [18]. Works related to functionalized GO nanocomposites used as platforms for drug delivery, cell imaging and interactions with biological systems have been reported [18,19]. Regarding the compatibility of graphene oxide with red blood cells, the results available in the literature are still controversial. There are works regarding graphene oxide exhibiting a low hemolytic effect [20,21] and additional published studies reporting GO with a high potential for hemolysis [22,23]. It is known that the synthetic parameters are related to the physicochemical properties of the obtained graphene oxide (charge distribution, size, morphology, organic functions, etc.); these, in turn, seem to be related to the possibilities of interaction with the RBCs, explaining the divergence of the results in this area of study. In the mentioned works, most authors agree that one of the possible features of graphene oxide related to RBC lysis is the electrostatic attraction between the negatively charged hydroxyl (O^-) and carboxylic acid groups (COO^-) on the GO surface and the positively charged phosphatidylcholine groups on the red blood cell membrane. From the point of view of the interaction of graphene oxide with proteins, studies in the literature suggest a significant attraction of this nanomaterial to proteins [24–26], demanding efforts in the development of new GO-based nanomaterials due to the decreased interactions with said biomacromolecules.

Mesoporous silica nanoparticles (MSNs) are a group of nanomaterials exhibiting interesting physical-chemical features. More specifically, the presence of silanol groups, Si-OH, on the surface allows several functionalization strategies with key molecules for the acquisition of new and important properties; in addition, the fact that these nanocarriers have high surface areas ($>900\ m^2\ g^{-1}$) and high pore volume ($>0.9\ cm^3\ g^{-1}$) allows the incorporation of guest molecules into their mesopores [27–30]. The interior of the nanoparticles can be functionalized with hydrophobic organic groups that substantially interact with antitumor drugs (which are mostly hydrophobic in nature), ensuring their retention and physico-chemical integrity up to release. Hydrophilic organic groups, important for colloidal stability in biological fluids such as blood (also hydrophilic) [31] and for covalent bonding on support nanomaterials such as graphene oxide, can also be grafted onto the external surface. Within the group of key molecules on the external surface of MSNs for the acquisition of new important biological properties, special attention is focused on hydrophilic organosilanes and polymers for colloidal stability, ligand molecules for selectivity for cancer cells and molecular markers for cell imaging [18,19,32]. Although considered safe by the US Food and Drug Administration (FDA), silica nanoparticles deserve attention in the context of their toxicity to RBCs. It is known that the significant chemical affinity of the silanol groups to the phosphatidylcholine groups present on the RBC membrane is related to the high hemolytic effects and, therefore, associated with low biocompatibility. However, bare silica nanoparticles (before external functionalization) need to be subjected to a post-modification process or added to another nanomaterial to enable intravenous application. As reported, the functionalization of said nanoparticles allows the shieldingör the reducing of the silanol groups (negatively charged as SiO^- groups) to minimize the hemolytic effects [33]. Paula et al. [34] reported a study showing that hemolysis rates decreased for silica nanoparticles functionalized with amino groups due to the electrostatic repulsion between the positively charged NH_3^+ groups and phosphatidylcholine groups present on the red blood cell membrane (also positively charged). From the point of view of the interaction of silica nanoparticles with blood proteins, there are reports in the literature showing a significant interaction of these colloids with polypeptides due to the presence of surface electrostatic charges (negative or positive). In addition, hydrophobic, steric and hydrogen bonding effects are also other possible results of these attractive interactions since the proteins have structures covering charged species (negatively or positively), hydrophobic regions and groups capable of interacting by hydrogen bonds [34–36]. This range of interactive possibilities also occurs in the process of protein adsorption on the surface of graphene oxide, which also contains charged groups and hydrophobic regions.

Considering the inherent interactions of graphene oxide and silica nanoparticles themselves, the objective of the current work is the development of a novel nanocomposite based on GO and MSNs through the external functionalization of silica with positive and hydrophilic amino groups (minimization of SiO^- species) and their functionalization on the GO surface through the amidation reaction between the amine and the carboxylic acid species available, respectively, on the surface of these materials. Once obtained, the physicochemical properties of the nanocomposites were investigated and related to the biological effects assessed by hemolysis and human plasma protein corona assays. In this context, nanocomposites of graphene oxide and silica nanoparticles with high contents of hydrophobic phenyl groups (for future studies involving the encapsulation of hydrophobic drugs) functionalized on their inner surface, and hydrophilic amino groups functionalized on their outer surface, were developed for the first time. Considering also the studies involving materials of this nature, the interaction of nanocomposites to RBCs and proteins present in human blood was evaluated.

2. Experimental

2.1. Materials

Hexadecyltrimethylammonium bromide (CTAB, ≥98.0%), 3-aminopropyltriethoxysilane (APTES, 99.0%), *N*-hydroxysuccinimide (NHS, 98.0%), potassium persulfate ($K_2S_2O_8$, 99.0%) and phosphorus pentoxide (P_2O_5, 98.0%) were purchased from Sigma-Aldrich (USA). *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, ≥99.0%) was purchased from Fluka (Japan). Ammonium hydroxide (NH_4OH, 27.0%), natural graphite powder, hydrochloric acid (HCl, 36.5–38.0%), potassium