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Physicochemical characterization of functionalized-nanostructured-titania as a carrier of copper complexes for cancer treatment

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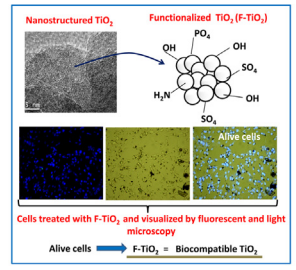
HIGHLIGHTS

- Nanostructured titania surface was functionalized with amino, sulfate and phosphate groups (F-TiO_2).
- F-TiO_2 was used as reservoir of copper complexes to obtain drug release systems.
- F-TiO_2 and copper complexes loaded on TiO_2 materials were characterized mainly by infrared and ultraviolet spectroscopies.
- The copper complexes maintained unchanged their structure after them were loaded on F-TiO_2.
- High biocompatibility of F-TiO_2 was observed after those different kinds of cells were treated with it.

GRAPHICAL ABSTRACT



Graphical Abstract. Surface's titania was functionalized with amino, sulfate and phosphate ions in order to obtain a biocompatible material.

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ABSTRACT

In the present paper we report the preparation and characterization of functionalized-TiO_2 (F-TiO_2) to obtain a biocompatible material to be used as carrier of alternative anticancer agents: copper acetate and copper acetylacetonate. The sol–gel procedure was used to prepare the fuctionalized titania material through hydrolysis and condensation of the titanium's butoxide. Sulfate, amine and phosphate ions served as functional groups which were anchored to the titania's surface. Mineral acids and gamma amine butyric acid were the precursors and they were added at the initial step of the synthesis. The copper complexes were loaded on titania and were also added to the reactor synthesis from the beginning. Infrared and ultraviolet–visible spectroscopies were the principal techniques used to the characterization of F-TiO_2 and copper complexes loaded on titania materials. Transmission Electronic Microscopy (TEM) was used to complement the characterization's studies. The biocompatibility of F-TiO_2 was evaluated by treating different cancer cell lines with increased concentration of this compound. The amine, the sulfate and the phosphate on the titania's surface, as well as the integral structures of the metal complexes on titania were well identified by infrared and ultraviolet–visible spectroscopies. The TEM photographs of $Cu(acac)_2$/F-TiO_2 and $Cu(Oac)_2$/F-TiO_2 materials showed the formation of

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nanoparticles, which have sizes ranging from 4 to 10 nm, with no morphology alterations in comparison with F-TiO_2 nanoparticles, suggesting that the presence of low quantities of copper do not affect the structure of the nanoparticles. The Energy Dispersive Spectroscopy (EDS) confirms the presence of copper on the titania's nanoparticles. The biological results indicate that there is more than 90% cell survival, thus suggesting that F-TiO_2 does not cause damage to the cells. Therefore, highly biocompatible titania was obtained by functionalizing its surface with those ions which in a certain way are similar to the hydrophilic heads of phospholipids in the double layer of the cell membrane.

1. Introduction

Nanomaterials are currently a focus of great interest, due to the fact that they constitute a new class of structural materials whose characteristics have shown either improved properties or even significantly different properties, when compared to their analogous coarse-grained materials [1]. The size reduction of the matter at the nanometric scale (1–100 nm) can be reflected in its improving physical properties such as strength, ductility, super plasticity at low and high temperatures, heat capacity, thermal expansion, high diffusion coefficients, and low melting points [1–3]. The materials at this scale can adopt a variety of shapes ranging from nano-tubes, nano-particles, nano-spheres, nano-fullerenes, nano-fibers to nano-ribbons [4–9].

Nanomaterials have opened a new era of technology which has had an impact on all sectors of human life: electronics and computers, mobile phones, food and agriculture industry, cosmetics, paints and, of course, health care. Specifically, its potential impact on health care is immense and pervades many aspects of the new area aptly labeled as nanomedicine [10]. Nanomedicine emerged at the end of the last century; since then, the discovery and design of new drugs, implantable materials and devices or molecular machineries on nanoscale dimensions introduced a complete renovation of controlled and targeted drug release, diagnosis, tissue replacement and/or surgical aids [1,11–13]. However, while nanomedicine can be considered a clinical reality, toxicity and biocompatibility of the nanomaterials have to be carefully evaluated, emphasizing on the understanding of the physicochemical properties that account for the adverse biological responses [11,14]. Biocompatibility refers to specific properties of a material not having toxic or injurious effects on biological systems. Biocompatible materials should not cause irreversible tissue damage, such as permanent tissue destruction, necrosis, significant fibrosis, and dystrophic calcification [15]. The biocompatible materials used in building nanomaterials include both natural and synthetic polymers such as chitosan, collagen, poly(ether) urethan, polyacrylamide/poly(acrylicacid), and N-isopropylacrylamide/acrylamide copolymers [16–20]. Inorganic nanomaterials such as metals (Au), inorganic oxides, carbon nanotubes (CNTs) [21–23], have gained interest in this field because it is possible to control their size, morphology, and surface chemistry which give them a wide variety of possible properties like biocompatibility with potential medical applications.

On the other hand, we have reported that with the surface's functionalization of metal oxides it is possible to obtain biocompatible materials [24–26]. We have determined that functional groups such as phosphate, sulfate, amine, can be anchored and generate positive and negative charges to the surface of the metal oxides. These charges can interact with the charges of the hydrophobic heads on the lipid bi-layer that conform the cell membrane. So the interaction between the charged oxide surface and the cell membrane does not result in the cells refusing the oxide. Because the cell accepts the oxide as part of itself, there is a high level of biocompatibility. We recently reported that F-TiO_2 had successfully acted as a carrier of copper complexes when they were released in different cancer cell lines [26]. Our research is focused on finding new drugs and new alternatives to administering these drugs in cancer treatment. We have found that copper acetate and copper acetylacetonate are highly toxic on different cancerous cells. We observed that the copper complexes alone and those loaded on nano-structured F-TiO_2 produced better cytotoxic effects on cancer cells than the cis-diamminedichloroplatinum (II) (Cis-Pt) compound. Cis-Pt is used to treat different types of cancer; however, despite its great efficiency, it generates severe side effects thus limiting its use.

As a continuation of a previous work, in the present work we are reporting the preparation and characterization of F-TiO_2 as carrier of copper complexes. The sol–gel process was used to prepare F-TiO_2 materials containing copper complexes and at the same time this process served us to disperse the copper complexes on the titania's matrix. We have used different spectroscopy techniques in order to analyze the surface of titania and determine the structure of the complexes in the final obtained materials. We also tested the biocompatibility of titania by treating several cancer cell cultures.

2. Experimental

2.1. Sample preparation

A biocompatible material was obtained by the functionalization of the titania's surface with sulfates, phosphates and amino functional groups. The functionalized-titania material was used as carrier of copper complexes

2.1.1. Functionalized-TiO_2 (F-TiO_2)

The functional groups precursors were sulfuric acid (REASOL, 95.98%), phosphoric acid (MONTERREY, 85%) and γ-aminobutyric acid (GABA) (SIGMA, 99%). The material was prepared according to the following process previously reported by López et al. [25,26].

One gram of GABA was dissolved in a mixture of 115 mL of ethyl alcohol (ALFIMEX, 96%) and 72 mL of deionized water under constant stirring. The mixture was refluxed at 70 °C and then 90 μL of sulfuric acid and 90 μL of phosphoric acid were added. Next, 70 mL of titanium n-butoxide (SIGMA ALDRICH, 97%) were slowly dropped-wise to the previous solution. After this point, the mixture was stirred and refluxed at 70 °C for 24 h. Once the gel was formed, the water and ethanol excess was removed and finally the resultant powder was dried at 70 °C during two days.

2.1.2. $Cu(Oac)_2$/F-TiO_2 and $Cu(acac)_2$/F-TiO_2 materials

The copper complexes used for this study were copper (II) acetate monohydrated [$Cu(Oac)_2$] (SIGMA-ALDRICH) and copper (II) acetylacetonate [$Cu(acac)_2$] (ALDRICH, 99.99%). The amounts used