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A new insight on nanomagnet–porphyrin hybrids for photodynamic inactivation of microorganisms

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ABSTRACT

Managing and securing access to clean water is a major global challenge. Traditional methods used in water treatment are based in disinfection processes that are toxic to several species, induce genetic damages on microorganisms and some of them are expensive and not always effective. Antimicrobial photodynamic inactivation (PDI) may represent a potential alternative to traditional methods. This approach refers to the combination of light, oxygen and a photosensitizer to induce microbial inactivation through oxidative stress, and is believed to be environmentally-friendly and economically feasible if the photosensitizer is immobilized into inert solid supports, which allows its recycling and reuse.

Previous results of our group, using a cationic porphyrin [5-(pentafluorophenyl)-10,15,20-tris(1-methylpyridinium-4-yl)porphyrin tri-iodide] coupled to cationized silica-coated magnetic nanoparticles of Fe_3O_4 (hybrid **6**), have demonstrated a remarkable antimicrobial activity for water disinfection through the photodynamic process. Herein, we extended our research to the synthesis of a new nanomagnet–porphyrin hybrid with a $CoFe_2O_4$ core (hybrid **7**) and we studied, for the first time, the recycling and reuse capability of this type of hybrids in water contaminated with bacteria. Bacterial inactivation was assessed by monitoring the bioluminescence of a Gram-negative bacterium (*Allivibrio fischeri*) during the photosensitization process.

Hybrids **6** and **7** showed to be photostable and maintained their magnetic properties during the photoinactivation experiments (irradiance of 40 W m^{-2} and a total light dose of 345 J cm^{-2}). The cumulative values of bacterial inactivation after a six-cycle reuse were ≈42 $\log_{10}$ colony-forming units (CFU) mL^{-1} in 21.5 h with hybrid **6** and ≈38 $\log_{10}$ CFU mL^{-1} in 27 h with hybrid **7**.

This study highlights the photostability and outstanding antibacterial properties of hybrids **6** and **7**, as well as their capability of being reused and potential of being recycled, which envisages a promising application in water disinfection.

1. Introduction

Water is an essential resource whose reuse starts to become an essential requirement. Even in developed countries, the treatment of drinking water and wastewater faces many challenges nowadays. The chlorine-based agents are still the most widely used for disinfection and although they are effective against bacteria and viruses, inexpensive and easy to use, the lack of effectiveness against parasites and the formation of toxic byproducts (e.g., trihalomethanes, haloacetic acids, bromate and chlorite) are major

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concerns. Alternatives to chlorine such as chloramines, chlorine dioxide, ozone and UV irradiation, have pros and cons with respect to cost, efficiency, stability, ease of application, and nature of formed by-products. Ozone is the most efficient disinfectant in terms of microbial inactivation but it is expensive, leads to toxic compounds and requires an *in situ* generation due to its unstable nature. Chloramines, although producing fewer by-products than chlorine, are less efficient. Chlorine dioxide is also an efficient disinfectant but it entails highly specialized operators, high cost and sometimes some odor and taste problems may occur. UV irradiation inactivates microorganisms that are resistant to chlorination but it does not offer residual protection [1,2].

In order to overcome the problems of microbial inactivation and formation of toxic by-products, new technologies have emerged. In recent years, nanotechnology has been identified as a promising alternative for the improvement of the water treatment process with useful features for different applications [3]. As regards disinfection and decontamination, bioactive nanoparticles (NP) have been developed [3], consisting of NP associated with, for example, photosensitizers (PS) that, in the presence of molecular oxygen and light, lead to the effective elimination of different biological entities. In this process, termed photodynamic inactivation (PDI), various processes of energy transfer and electronic state transition take place with further production of reactive oxygen species (ROS: singlet oxygen and/or free radical species). The aggressive action of these species, especially singlet oxygen, causes irreversible effects at the level of lipids, proteins and nucleic acids, leading to cell inactivation [4]. Furthermore, the development of resistance has not been observed in repeated cycles of PDI in semi-lethal conditions using tetrapyrrolic macrocyles [5–7].

The use of this approach in the environmental context has been explored by some research groups who believe it is an interesting alternative for the decontamination of wastewater [8–12] and water of fish farms [13–15]. The idea of using PDI for this purpose is intended to overcome economic, ecological and public health issues, with minimal environmental impact.

There is a myriad of aspects to consider before using PDI for water treatment, such as physical, chemical and microbiological properties of water [8,12–14]; turbidity (presence of suspended solids) [8,13]; thickness of the water column (related to the depth of light penetration); dissolved oxygen concentration [8]; light source and light dose [13,15–17]; irradiation time [8,12]; photophysical and photochemical properties of the PS [11,15]; and concentration of the PS [8,11–14], possibility of immobilization on solid supports [9,18]; and reuse of the PS.

Porphyrin-based PS have been commonly used in PDI, even in environmentally oriented approaches, specifically cationic *meso*-substituted compounds [8,9,11–17,19]. The potential benefits of this therapy include the use of sunlight as energy source (the most economic option) [13,16,20]; the decreased or absent toxicity of the PS, specifically porphyrins, towards higher organisms at photochemically active doses [15]; the gradual photobleaching of porphyrins by solar light [21], which prevents their accumulation in the environment; the reuse of the PS [9]; and the possibility of water recycling after disinfection (for agricultural and landscape irrigation, for example) [12].

In order to achieve the actual field implementation and to diminish the potential environmental impact, PS have been covalently linked/grafted or incorporated into inert solid supports such as silica powders or beads [22], chitosan membranes [9], chitosan NP [23], silica-coated magnetite NP [24] or multi-walled carbon nanotubes [25], and tested upon Gram-negative bacteria, bacteriophages, yeasts and viruses, with significant antimicrobial activity. The immobilization of the PS aims to allow both the efficient elimination of microorganisms during several cycles of use and the complete PS removal from the treated medium.

The synthesis of different silica based nanomagnet–porphyrin hybrids and their use in PDI of *Escherichia coli* and T4-like bacteriophages has been described [24]. These hybrids consist of porphyrin covalently linked to silica-coated iron oxide (Fe_3O_4) NP. The major advantages of these NP is their magnetic behavior provided by the metal core [26], the prevention of the intrinsic tendency of aggregation and oxidation by the silica shell, as well as the ability to be functionalized [27] with a very effective broad-spectrum PS [13,14,16,28–30]. The PDI with the cationic porphyrin [5-(pentafluorophenyl)-10,15,20-tris(1-methylpyridinium-4-yl)porphyrin tri-iodide] **1a** coupled on cationized NP (hybrid **6**) led to a reduction in viability of 4.8 $\log_{10}$ of *E. coli* at 20 μM (PS concentration in the hybrid) and 43.2 J cm^{-2} (180 min of irradiation with white light at an irradiance of 40 W m^{-2}), and 6.8 $\log_{10}$ of T4-like phage at the same concentration and 14.4 J cm^{-2} [24].

The promising results obtained with hybrid **6** (Fe_3O_4) in biological experiments led us in to synthesize a new hybrid (**7**) with a cobalt iron oxide core ($CoFe_2O_4$), less susceptible to be oxidized. The ability of hybrids **6** and **7** ($CoFe_2O_4$) of being recycled and reused in bacterial photoinactivation was evaluated for the first time. The marine bacterium *Aliivibrio fischeri* was used as a model of Gram-negative bacteria. Being a naturally bioluminescent bacterium (emits green light at ≈490 nm), its viability can be quickly and easily monitored during the laboratory PDI tests using a luminometer that records the light emission/light loss in real-time, instead of using the conventional time-consuming pour plating method.

2. Experimental

2.1. Synthesis of the porphyrin photosensitizer

The synthesis and the full characterization of 5-pentafluorophenyl-10,15,20-tri(4-pyridyl)porphyrin (**1**) and of the corresponding cationic 5,10,15-tris(1-methylpyridinium-4-yl)-20-(pentafluorophenyl)porphyrin tri-iodide (**1a**, Scheme 1) were already described in the literature [10,24,31].

2.2. Preparation of the cationic nanomagnetic material ***8*** and the corresponding nanomagnet–porphyrin hybrid ***6***

These Fe_3O_4 silica core shell materials were prepared according to the procedure described previously by us [24].

2.3. Preparation and characterization of magnetic silica nanoparticles ***3***

The $CoFe_2O_4$ silica core–shell NP **3** was prepared by the conventional co-precipitation method [32], where $CoCl_2 \cdot 6H_2O$ (0.24 g) and $FeCl_3$ (0.33 g) were dissolved in deionized water (10 mL at 80 °C) with vigorous stirring. Then, NaOH (1.20 g) was slowly added, forming immediately black particles. The resulting mixture was kept under stirring at 70–85 °C for 1 h and then washed twice with deionized water. Sodium metasilicate (7.92 g) was dissolved in deionized water and the pH value of the solution adjusted to 12–13 by addition of concentrated hydrochloric acid (37%). The sodium metasilicate solution and the prepared $CoFe_2O_4$ nanocores were poured into a beaker equipped with a mechanical stirrer and heated to *ca.* 70 °C. The mixture was ultrasonicated for 15 min and then, the temperature was increased to 75–80 °C. A HCl solution was added dropwise to adjust the pH value to 6–7. The particles formed were washed several times with deionized water and then