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# Silica nanoparticles doped with anthraquinone for lung cancer phototherapy

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ABSTRACT

In the present study, $SiO_2$ nanoparticles functionalized with 3-(2-aminoethylamino)propyl group (SiNP-AAP) were used, for the first time, to covalently bond rose bengal (SiNP-AAP-RB) or 9,10-anthraquinone-2-carboxylic acid (SiNP-AAP-OCAq). The functionalized SiNP were characterized by: Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM); elemental analysis (CHN) for determination of the dye concentration; FTIR and UV–vis diffuse reflectance (DR-UV–vis) and a surface area study (BET). The functionalized SiNPs were applied in photodynamic therapy (PDT) against lung cancer cell lines. The evaluated cytotoxicity revealed 20–30% cell survival after 15 min of PDT for both materials but the OCAq concentration was half of the RB nanomaterial. The phototoxicity was mainly related to oxidative stress generated in the cellular environment by singlet oxygen and by hydrogen abstraction as confirmed by the laser flash photolysis technique. The unprecedented results indicate that SiNP-AAP-OCAq is a possible system for promoting cell apoptosis by both type I and type II mechanisms.



## 1. Introduction

During the last decades, photodynamic therapy (PDT) has been proposed as an innovative treatment for cancer and since then, many nanoapproaches have been developed based on light induced conversion of energy by quantum dots for elimination of bacteria [1] or cancer cells [2], and different nano-drug-carriers such as: liposomes [3], nanosized silica, nanotubes [4] and nanodiamonds [5]. Due to the high specificity, the biological consequence of PDT is the localized oxidative damage inflicted by the photochemical process initiated by the photosensitizer [6]. As the chemical effects induced by photochemical processes can be better controlled, PDT has become a State-of-the-Art alternative to conventional invasive treatments for cancer [7].

The use of PDT is based on the action of singlet oxygen ($^1O_2$), a reactive oxygen species which is generated by energy transfer from the $T_1$ state of a photosensitizer to intracellular oxygen upon illumination of the photosensitizer at an appropriate wavelength (type II mechanism) [8]. This kind of mechanism appears to be the most relevant in biological systems [6]. Once formed, this highly reactive species has the ability to attack biological systems within a short period of time and can result in reduced side effects [9].

Although PDT points to a promising strategy for clinical therapy, the photosensitizer toxicity and solubility restricts its application [10]. To the best of our knowledge Photofrin® has been used in PDT in clinic chemotherapy of different cancer types such as esophageal, lung, bladder, gastric and cervical [11]. Since the beginning of photodynamic therapy, other PDT drugs have been approved by the FDA: Foscan®, Visudyne®, Levulan®, Metvix® [12]. The latter two drugs are not photosensitizers but are converted by the body to protoporphyrin IX (or the methyl derivative for Metvix) *via* the heme biosynthetic pathway [13]. In order to overcome these problems, chemists have focused on developing novel PDT strategies. One interesting approach is based on drug encapsulated targeted nanoparticles as therapeutics with the potential to improve efficacy and safety of currently available drugs.[7] Recently, efforts aiming to develop a new group of photodrugs, anionic phorphyrin grafted to porous silicon nanoparticles, resulted in improved uptake by tumor cells. The use of functionalized silicon nanoparticles improved the bioavailability of the photosensitizer and resulted in enhanced photoactivity [14–17].

Aromatic ketones (quinones) are a group of molecules that have been extensively used as anti-malaria drugs [18,19], for the treatment of multiple sclerosis [20,21], as laxatives [22] and as chemotherapeutic agents [23–25]. Electronic excitation of the ketone ground state results,

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after intersystem crossing, in the respective triplet excited state which in the presence of oxygen can generate $^1O_2$ or abstract hydrogen from suitable donors such as DNA (the latter process being a type I mechanism) [26–29]. Although these molecules have well defined photochemistry, there are few studies combining nanotechnology with quinones for cancer PDT. So, it's interesting to evaluate the photoactivity of quinones with cancer cells in order to develop new drugs for PDT.

In the present study, rose bengal (RB) and 9,10-anthraquinone-2-carboxylic acid (OCAq) were chemically bonded to the surface of silica nanoparticles functionalized with 3-(2-aminoethylamino)propyl group and the phototoxicity of these two materials were evaluated under visible light irradiation using the lung cancer cell line A549 as a prototype test.

## 2. Experimental Section

### 2.1. Organic Functionalization of $SiO_2$ Nanoparticles With Dyes

Rose bengal and anthraquinone-2-carboxylic acid were purchased from Sigma-Aldrich and used as received.

The SiNP-AAP was prepared according to the following procedure: 15 g of nanometric silica (99.5%, Aldrich) with average size varying from 5 to 15 nm were submitted to drying for 12 h at 160 °C and then at 160 °C for 6 h under $10^{-3}$ Torr. After the drying period, the nanoparticles were functionalized with [3-(2-aminoethyl amino)propyl]trimethoxysilane (AAPTMS, $(CH_3O)_3Si(CH_2)_3NHCH_2CH_2NH_2$) as detailed elsewhere [30]. The dried $SiO_2$ nanoparticles were mixed with AAPTMS (15 mL) in dry toluene (100 mL). The reaction was maintained at 100 °C for 24 h under constant stirring in a nitrogen atmosphere. The amino-functionalized silica (SiNP-AAP) was thoroughly washed in a soxhlet extractor during 6 h with ethanol and then vacuum dried ($10^{-3}$ mm of Hg) at 60 °C for 4 h [15].

After functionalization of the support, two materials were prepared by covalently bonding rose bengal (RB) and anthraquinone-2-carboxylic acid (OCAq) to the SiNP-APP surface. Initially, the carboxyl group of both photosensitizers was transformed into the respective acyl chloride by refluxing the dyes with thionyl chloride for 2 h. Excess thionyl chloride was removed by evaporation under reduced pressure and any remaining gases from the reaction were removed under a nitrogen stream. Subsequently, 0.46 g of SiNP-AAP was added to a solution of the dye in anhydrous chloroform (1 mmol/20 mL). The reaction medium was refluxed for 4 h and then stirred at room temperature for another 20 h. The immobilized materials were centrifuged at 5000 rpm for 5 min and the solvent decanted. In order to remove non-bonded dyes the materials were washed with chloroform (5 mL) and re-centrifuged [31]. This procedure was repeated until no dye could be detected in the chloroform washings by UV–vis. The materials were named SiNP-AAP-RB and SiNP-AAP-OCAq.

### 2.2. Characterization of Silica Nanoparticle Materials: Physicochemical Analysis

Scanning electron micrographs of the SiNP and SiNP-AAP materials were obtained by dispersing the samples on double-sided conductive tape on a gold support and coated with gold before the experiment. SEM images were acquired using a JEOL model JSM 6460LV scanning electron microscope at an acceleration voltage of 30.0 kV (Tokyo, Jeol) and 30,000× magnification.

Samples of SiNP-AAP-RB and SiNP-AAP-OCAq materials were dispersed on water and dried at room temperature and were put overhead of grade copper covered for Formvar on Tecnai Spirit microscopy (FEI company) 120KV for obtain the images of Transmission electronic micrographs.

Analysis of the surface area of the SiNP and SiNP-AAP was performed using the multipoint BET method. Nitrogen adsorption experiments were performed on a Quantachrome Nova Model 1200E coupled to an automatic nitrogen gas adsorption instrument (Boynton Beach, FL, USA).

The carbon, hydrogen and nitrogen elemental composition of the SiNP-AAP, SiNP-AAP-RB and SiNP-AAP-OCAq were determined using an Elemental Analyzer (Perkin Elmer 2400 SERIES II). This measurement was done in triplicate and allowed determination of the concentration of the dyes on the surface of the materials.

The infrared (IR) analyses were performed using a NICOLET Magna–IR 760 spectrophotometer with 4 $cm^{-1}$ resolution and range from 4000 to 400 $cm^{-1}$. The samples were analyzed in KBr pellets at room temperature.

The UV–vis diffuse reflectance spectra were measured using a UV-2450 SHIMADZU spectrometer with barium sulfate as standard. Samples were scanned from 700 to 250 nm using 1 mm thickness quartz cells filled with the samples. The molar extinction coefficient for RB was 161,300 (560 nm) L $mol^{-1}$ $cm^{-1}$ and for OCAq was 2311 (330 nm) L $mol^{-1}$ $cm^{-1}$. The singlet oxygen ($^1O_2$) detection was performed by following its phosphorescence signal, both as its decay lifetime (at 1270 nm) as well as by its emission spectrum (from 1200 to 1300 nm). Both experiments were conducted in a FL900 spectrofluorometer from Edinburgh Instruments coupled to a NIR PMT from Hamamatsu Model H10330-45. Both experiments were performed using a CryLas LASER Model FTSS 355-50 with 100 μJ/pulse and 5 ns (duration) to pump the molecules. All the measurements were done in the solid state using the front face scheme. The singlet oxygen quantum yield was determined by using a variation of the Gao methodology [32]. All samples (rose bengal, SiNP-AAP-OCAq, SiNP-AAP-RB and acid anthraquinone) were solubilized in acetonitrile and absorbance was fixed at 0.8. The excitation beam intensity was ranged by using neutral density filters with optical density varying from 0.1 to 0.9. All samples were correlated to rose bengal, using $\Phi_{\Delta RB} = 0.54$. The nanoparticle singlet oxygen quantum yield was calculated according to Eq. 1.

$$\Phi_{\Delta NP}/\Phi_{\Delta RB} = I_{NP}/I_{RB} \quad (1)$$

Where $\Phi_{\Delta NP}$ and $I_{NP}$ are the singlet oxygen quantum yield and singlet oxygen signal intensity for the SiNP-AAP-OCAq and SiNP-AAP-RB materials, while $\Phi_{\Delta RB}$ and $I_{RB}$ are the singlet oxygen quantum yield and singlet oxygen signal intensity for the rose bengal reference solution.

The laser flash photolysis (LFP) experiments were carried out on a LuzChem Instrument model mLFP122. Samples were contained in a 10 mm × 10 mm cell made from Suprasil tubing and were de-aerated by bubbling with argon for about 20 min. The samples were irradiated with the third harmonic of a Nd/YAG Surelite laser ($\lambda = 355$ nm). All laser flash photolysis experiments were performed in acetonitrile suspension. The concentration of SiNP-AAP-OCAq was adjusted to yield an absorbance of ~0.3 at the excitation wavelength (355 nm). The rate constants for the reaction of triplet SiNP-AAP-OCAq with the different quenchers employed in this work were obtained from Stern–Volmer plots, following Eq. 2.

$$k_{obs} = k_o + k_q[Q] \quad (2)$$

where: $k_o$ is the triplet decay rate constant in the absence of quencher; $k_q$ is the triplet decay rate constant in the presence of the quencher and [Q] is the quencher concentration in mol $L^{-1}$. The decay trace at 390 nm was used to determine the quenching rate constants.

### 2.3. Cell Culture

The human lung adenocarcinoma cell line, A549, was cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10%