Mesoporous silica nanoparticles to increase the photodynamic activity of zinc phthalocyanine and rose bengal

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***Abstract*— Mesoporous silica nanoparticles (MSNs) exhibit unique properties such as biocompatibility, high surface area, and adjustable pore size, which make them ideal carriers for therapeutic agents. In light-based therapies, such as photodynamic therapy (PDT) and photothermal therapy, MSNs provide further advantages over other drug delivery vectors due to their optical transparency. Previous research has also shown that incorporating photosensitizers into MSNs can enhance their photodynamic activity. The current study discusses the mechanisms by which MSNs increase photodynamic activity and investigates this phenomenon by comparing the performance of two photosensitizers, zinc phthalocyanine (ZnPc) and rose bengal (RB) in terms of singlet oxygen production when integrated into MSNs. According to our results, both MSN-incorporated PSs used in this study produced more singlet oxygen compared to their free forms.**

Keywords—photodynamic therapy; zinc phthalocyanine; rose bengal; drug delivery; mesoporous silica nanoparticles.

I. INTRODUCTION

PDT, investigated as an alternative to conventional methods in cancer treatment, uses light and a light-sensitive chemical (photosensitizer, PS) to kill cancer cells selectively [1-3]. An effective PDT requires localizing the PS molecules in the tumor tissue, especially in malignant cells before irradiation. The PSs should also be soluble in biological media for systemic administration. Hydrophobic PSs can easily penetrate cellular membranes but tend to self-aggregate in aqueous solutions. Coupling the PS molecules with a vector, such as a liposome or a nanoparticle can decrease self-aggregation. On the other hand, the intracellular accumulation of hydrophilic PSs can be poor, which can also be increased by coupling them with such vectors [4-8]. Mesoporous silica nanoparticles (MSNs), highlighted in the literature for their easy synthesis, large surface area, and biocompatibility, are among the nanoparticles being researched for this purpose [9, 10]. MSNs are also advantageous in PDT for their optical transparency [6]. Moreover, the singlet oxygen production capacity of PSs can be increased when incorporated with MSNs [6, 8].

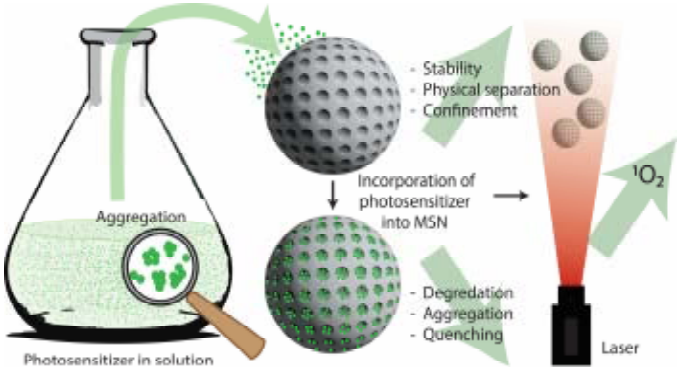


Fig. 1: Incorporation of PS molecules with MSNs decreases their degradation, aggregation, and quenching. Moreover, the physical separation and confinement of MSN-incorporated PS molecules lead to increased singlet oxygen production.

This study focuses on the phenomenon that the incorporation of the PSs with MSNs increases their photodynamic activity, by comparing the change in the singlet oxygen production of two MSN-incorporated PSs, RB and ZnPc, and discussing the potential reasons for this outcome.

II. METHODS

A. Synthesis of nanoparticles

PSs and chemicals necessary for nanoparticle synthesis were purchased from Sigma-Aldrich. To synthesize MSNs, distilled water was first mixed with ammonium-hydroxide (NH_4OH) on a magnetic stirrer and then, cetyltrimethylammonium-bromide (CTAB) was added into the mixture as described previously [11]. Subsequently, tetraethyl-orthosilicate (TEOS) and ethanol were added, and the solution was mixed at 60°C for 2 hours before the final washing and dispersing steps. MSNs were then either amino-functionalized using (3-Aminopropyl)triethoxysilane (APTES) or no further functionalization was performed before PS incorporation. In the APTES functionalized group, 12 µL of APTES was slowly added to MSNs that were dissolved in ethanol, and the mixture

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was stirred in the magnetic for 24 hours. To incorporate PS molecules with MSNs, the nanoparticles were dissolved in ethanol and mixed with PSs. The mixture was finally stirred at room temperature for 24 hours.

B. *Characterization of the nanoparticles*

The size and shape of the synthesized nanoparticles were determined using a Thermo Scientific Quattro S SEM scanning electron microscope. The absorption spectra of the nanoparticles were obtained using a Nanodrop 2000c UV–visible spectrophotometer.

C. *DPBF Analysis*

The production of singlet oxygen by irradiation of free PSs and PS-MSNs was compared using a protocol involving 1,3-Diphenylisobenzofuran (DPBF). Briefly, DPBF and PSs are dissolved in appropriate solvents (ethanol for RB, dimethyl sulfoxide for ZnPc), then the solution was exposed to light that excites the PSs. In this study, RB and MSN-RB were irradiated using a 530 nm light source. In ZnPc experiments, the samples were irradiated using a 660 nm light source. Both light sources were used at 100 mW/cm² power density. The assay relies on the interaction of singlet oxygen molecules generated during the irradiation of PSs, leading to a decrease in its absorbance peak of DPBF at 410 nm [12]. Thus, the rate at which this peak diminishes can be used to measure the singlet oxygen production of the PDT agents. In this study, previously determined concentrations of PSs and PS-MSNs were incubated with 50 µM DPBF. Absorbance values were recorded using a UV-visible spectrophotometer at 410 nm after irradiations with light sources. The acquired data were normalized compared to the "no irradiation" group.

III. Results

A. *Morphology of the nanoparticles*

The scanning electron microscope images show that the nanoparticles were spherical, and the diameter of the nanoparticles was around 50 nm.

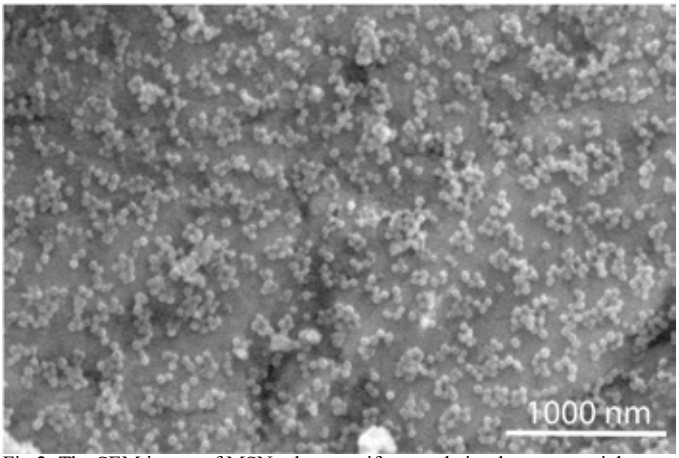


Fig 2. The SEM image of MSNs shows uniform and circular nanoparticles.

B. *Results of the DPBF analysis*

The comparison of singlet oxygen production of free PS and MSN-PS was performed using the DPBF assay. Figure 3 shows that the absorbance of DPBF at 410 nm decreases with repeated irradiation for all samples, which can be a surrogate for singlet oxygen production of the PSs or MSN-incorporated PSs because DPBF is known as a singlet oxygen quencher. When the two curves in Figure 3a are compared, the sharper drop in the MSN-RB curve (blue) indicates that the singlet oxygen production of MSN-incorporated RB was higher than the free RB (orange). In the ZnPc-mediated experiments on the other hand, although the curves were close to each other, the drop in the MSN-ZnPc (red) was again higher than the free ZnPc (green). Therefore, in both cases, the MSN-incorporated PSs produce more singlet oxygen compared to their nonconjugated forms, which is consistent with the literature [9, 13, 14].

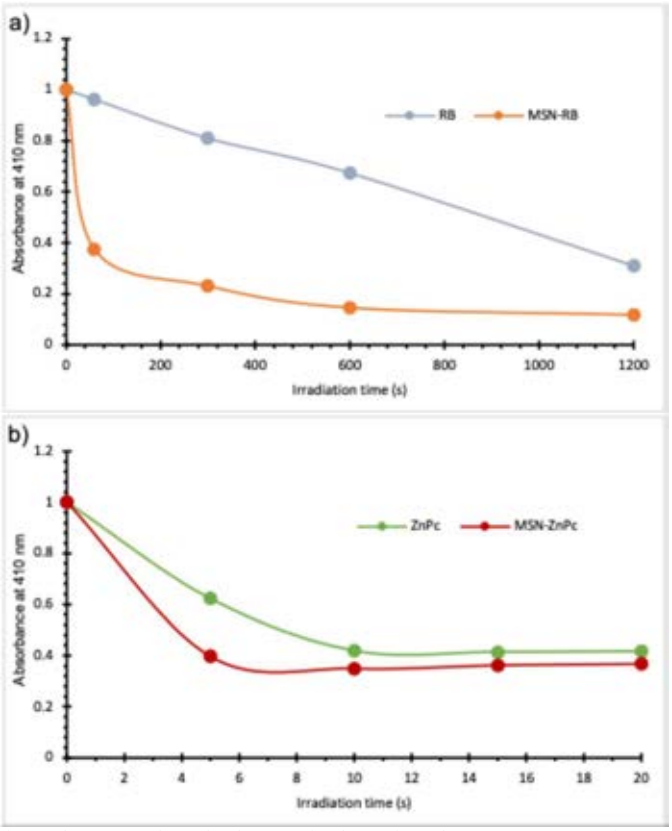


Fig 3. The curves show the decrease in absorption of DPBF at 410 nm after repeated irradiations.

IV. Discussion

PDT is based on the principle that photosensitizers absorb and get excited by light in the visible region, which results in the production of reactive oxygen species that are toxic to cells. Drug delivery systems are primarily used to enable the delivery of hydrophobic molecules into the bloodstream and to allow hydrophilic molecules to pass through the cell membrane. Specifically for photodynamic therapy, it is known that drug delivery systems also have benefits such as preventing the reduction of the photochemical and photophysical properties of photosensitizers [15]. For example, photosensitizers tend to aggregate in solution, which disrupts their monomeric form, and therefore their photodynamic efficacy [4, 6, 8]. Additionally, PS molecules introduced into the bloodstream can degrade until reaching the tumor. Incorporation with MSN can provide physical separation between PS molecules and make them

confined within the pores of the MSN. Thus, the PSs can remain in monomeric form and increase their concentration in a small area. Additionally, PS molecules within the MSN pores are protected against degradation. This can mean a reduction in aggregation and degradation, along with the preservation or even enhancement of photodynamic activity.

When the results of the DPBF experiment are examined, the following comments can be made:

- The combined states of both PSs with MSN produce more singlet oxygen comparing their free forms.
- In experiments using RB (Figure 3a), the degradation of DPBF occurs much slower comparing experiments using ZnPc (Figure 3b). The two PSs have similar singlet oxygen quantum yields, therefore, this difference should not be due to the difference in singlet oxygen quantum yield [16]. The concentration of ZnPc loaded per MSN is higher compared to RB, which can be the reason for this difference (data not shown here but will be presented in detail in a forthcoming manuscript). Furthermore, since one of these PSs dissolves well in ethanol and the other in DMSO, the experiments were carried out in these two different solvents. As it is known that singlet oxygen production varies with solvent, the difference in the protocol can be considered as one of the reasons for the difference in singlet oxygen production.
- As shown in Figure 3a, the singlet oxygen production of RB with and without MSN was highly different from each other. The long-term occurrence of degradation in the experiment with RB might allow us to observe this effect more clearly. On the other hand, the high singlet oxygen production in the MSN-RB group might be due to the PS being bonded to MSN through APTES since this surface modification method is known to prevent aggregation [17].
- In the ZnPc-mediated experiment, it is observed that no matter how long the samples are irradiated, some absorbance remains at 410 nm. This is not due to incomplete DPBF degradation but because ZnPc has minor absorbance at that wavelength.

In summary, the results of this showed that incorporation with MSN may increase the singlet oxygen production of PSs. Future studies will evaluate the same effect in other PSs, as well as the *in vitro* photodynamic activity of the free and MSN-incorporated PSs.

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