A Multifunctional Theranostic Nanoplatform for Image-Guided Surgery and Intraoperative Therapy

O. R. Taratula, C. Schumann, T. Duong, K. L. Taylor, and O. Taratula

Department of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, Portland, Oregon 97225, United States, Oleh.Taratula@oregonstate.edu

ABSTRACT

This work is focused on the development of a multifunctional agent that, when injected, can accumulate at the cancer site and help surgeons see non-visible tumor cells during surgery. Through the addition of light irradiation, the same agent can be used immediately after surgery to kill off any hidden cancer cells that may have been left behind. A single naphthalocyaninebased theranostic nanoplatform, therefore, was developed for concurrent NIR fluorescence imaging and combinatorial phototherapy with dual photodynamic (PDT) and photothermal (PTT) therapeutic properties . The conversion of a hydrophobic SiNc into a biocompatible nanoplatform (SiNc-NP) was realized by SiNc encapsulation into the hydrophobic interior of a polypropylenimine (PPI) dendrimer following surface modification with polyethylene glycol (PEG). The efficiency of SiNc-NP as an NIR imaging and phototherapeutic agent was confirmed in vitro and in vivo.

Keywords: naphthalocyanine, photothermal therapy, photodynamic therapy, near-infrared imaging, theranostic

1 INTRODUCTION

Each year there are about 22,000 new cases of ovarian cancer in the USA, and more than 14,000 women die because of it [1]. The high mortality rate is attributed to the fact that 60% of cases are diagnosed at an advanced stage, after the cancer has spread to the abdominal cavity and adjacent organs. Currently, optimal surgical resection of all disease sites is the only way to improve survival of these patients. Even with the best microsurgical techniques, however, surgeons, using visual inspection for malignant tissue identification, leave behind microscopic tumors that lead to cancer relapse. If these tumors could be detected, resected and treated more efficiently during surgery, survival rates would significantly increase. It is important, therefore, to improve tumor delineation during surgery enhancing surgical resection efficiency and provide an effective intraoperative treatment, to further eliminate any unresectable tumors. A recent clinical trial proved that the number of ovarian cancer tumors resected by surgeons under the guidance of a visible fluorophore (FITC) increased by 5.3-fold [2]. However, FITC is not optimal for surgery because high absorption and scattering of visible light by body tissue allow detection of only the surface of the tumors; and, importantly, high autofluorescence from healthy tissue reduces contrast. Since biological tissue demonstrates minimal autofluorescence and light absorption in the NIR spectral region (750 – 900 nm), NIR fluorophores can overcome these challenges [3]. The available NIR fluorophores, like indocyanine green (ICG), however, are not cancer specific and undergo irreversible photobleaching under exposure to light, which would give false negative results to the surgeon [4].

Therefore, it is critical to develop a cancer-targeted nanomedicine platform that enables maximal surgical resection by optically imaging tumor margins and can be simultaneously employed intraoperatively as a cancer therapeutic agent. The main building block of our delivery system is a photosensitizer (naphthalocyanine, SiNc) that upon exposure to NIR light generates strong NIR fluorescence signal for imaged guided surgery and destroys cancer cells by producing intercellular reactive oxygen species (ROS) and heat via PDT and PTT therapeutical mechanisms respectively. In vitro and in vivo studies of the developed SiNc-NP are discussed.

2 RESULTS AND DISCUSSION

Here, we employ silicon naphthalocyanine (SiNc) as the main building block to prepare the single agent-based nanomedicine platform (Fig. 1).

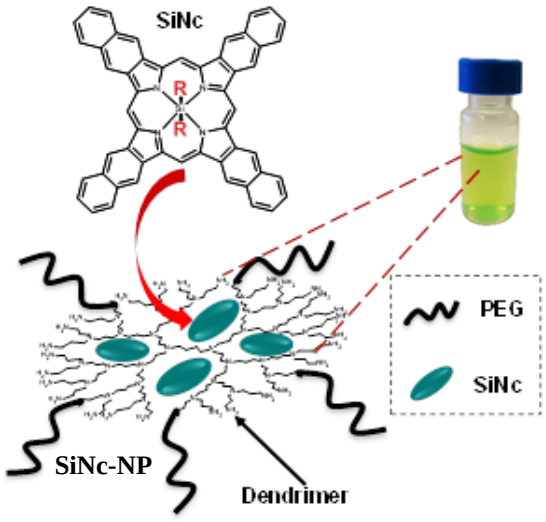

Figure 1: Nanomedicine platform (NP) based on SiNc-loaded dendrimer. The inset: SiNc-NP solution in water.

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2.1 Nanometer-Sized Complex

Owing to its unique intrinsic properties, including enhanced absorption in the spectral range 750 - 800 nm, SiNc can be efficiently activated with NIR light to produce fluorescence, ROS and heat for imaging and combinatorial treatment of tumors [5]. However, potential clinical application of SiNc is substantially limited by poor water solubility, severe aggregation and lack of cancer specificity [5]. Particularly, an intrinsic tendency of SiNc molecules to aggregate in aqueous environment, through π-π stacking and hydrophobic interactions, results in the self-quenching effect of the excited states [6]. Consequently, after exposure to NIR light, aggregated SiNc molecules can only dissipate the absorbed energy through nonradiative decay (heating), while fluorescence (imaging) and ROS production (PDT) are diminished [7]. To attain water solubility and preserve fluorescence, ROS and heat generation properties, we encapsulated SiNc molecules into the interior of the water-soluble polypropylenimine (PPI) dendrimers (Fig. 1). The specific structure of PPI dendrimers, having a combination of interior hydrophobic pockets and peripheral hydrophilic primary amines, offers the possibility to encapsulate and separate the SiNc molecules thereby both decreasing aggregation and enhancing water solubility. Further, to enhance stability and biocompatibility of the dendrimer surface of the SiNc-PPI was modified with a hydrophilic polymer (polyethylene glycol, PEG). The PEG layer enhances water solubility, minimizes aggregation, prevents the immune system recognition , decreases toxicity, and leads to improved tumor accumulation via EPR effect [8].

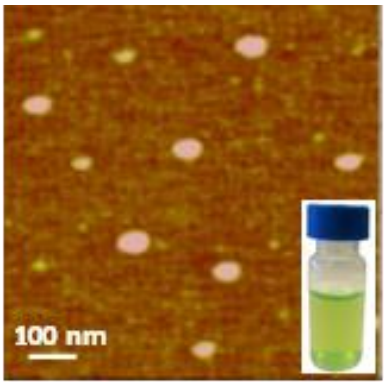

Figure 2: AFM image of the SiNc-NP.

The encapsulation procedure is based on a simple mixing of SiNc dissolved in THF with methanol solution of PPI dendrimers. After evaporation of the organic solvents, resultant complexes can easily be dissolved in water, providing SiNc aqueous solubility of at least 1 mg/mL. We also discovered that modification of SiNc with hydrophobic organic linkers (R) improves solubility in organic solvents, thus enhancing loading efficiency into the hydrophobic interior of the dendrimer up to 20 %. According to AFM images (Fig. 2), the dendrimer-based nanoplatform loaded with SiNc has a diameter of 64.6 ± 9.1 nm, which is within the desired range of 10 − 100 nm to prevent elimination by the kidneys (>10 nm), recognition by macrophage cells (<100 nm), and to enhance tumor-targeted delivery via the EPR effect (<200 nm) [9].

2.2 NIR Fluorescence Imaging

The SiNc(R) encapsulated in the dendrimer-based nanocarrier exhibited a strong NIR absorption in water with an absorbance peak at 782 nm (Fig. 3A, black curve).

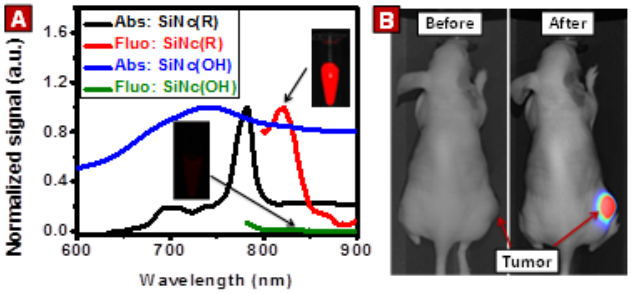

Figure 3: (A) Normalized absorbance (Abs) and fluorescence (Fluo) spectra of substituted SiNc(R) and non-substituted SiNc(OH) encapsulated in PPI dendrimer. Insets indicate fluorescence images of SiNc(R) and SiNc(OH) complexes in water recorded with Li-COR Pearl Animal Imaging system. (B) Images of mouse with ovarian tumor before (left) and after (right) injection with SiNc-NP.

The detected absorption fits within the NIR optical window (750-900 nm) required for an efficient fluorescence imaging and phototherapy [3]. Importantly, upon excitation with NIR light, the developed complex showed distinctive fluorescence emission at 820 nm in aqueous media (Fig. 3A, red curve and inset). In contrast, no detectable fluorescence signal was observed for the complexes based on unsubstituted SiNc(OH) (Fig. 3A, green curve and inset), which indicates the formation of non-fluorescence aggregates during the encapsulation process. Efficiency of SiNc(R) complexes as an NIR-imaging agent was confirmed in vivo by recording the strong fluorescence signal in the tumor area (Fig. 3B, right image). No signal was detected in the mouse before complex injection (Fig. 3B, left image), revealing that the recorded signal is not related to tissue autofluorescence. Our data revealed that encapsulation of substituted SiNc(R) within the PPI dendrimer interior decreases aggregation and preserves fluorescence intensity after dispersion of the nanocarriers in water.

2.3 Heat and ROS Generation

We have revealed that only a nanoplatform based on substituted SiNc(R) can simultaneously activate both PTT (heat) and PDT (ROS) mechanisms in ovarian cancer cells

exposed to 785 nm laser light of 1.3 W/cm^2 power density. The SiNc(R)-treated cancer cells exhibit rapid heating to 52 ^{o}C upon exposure to NIR light (Fig. 4A, black curve). In contrast, the temperature change for non-transfected cells was less than 1 °C under the same experimental conditions (Fig. 4A, red curve), indicating that 785 nm laser (1.3 W/cm^2) alone is not capable of heating. We have also confirmed efficient intracellular ROS generation by the substituted SiNc(R)-based nanoplatform upon NIR light activation. The cancer cells incubated with the SiNc(R) nanoplatform, then subsequently irradiated with the NIR laser light (785 nm, 1.3 W/cm^2), showed a 6-fold elevation in intracellular ROS levels, compared to the controls (Fig. 4B). In contrast, the unsubstituted SiNc(OH) encapsulated in the dendrimer was not able to increase intracellular ROS levels significantly under the same experimental conditions, which is related to aggregation of SiNc(OH) during the encapsulation process and the quenching of the excited states.

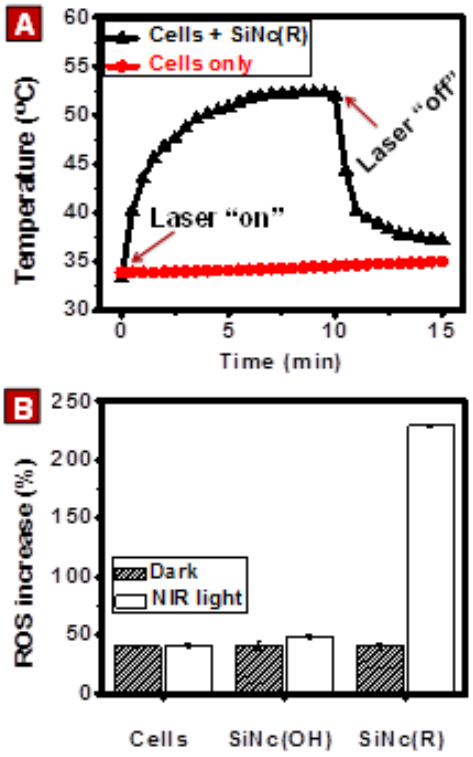

Figure 4: (**A**) Temperature profile of A2780/AD ovarian cancer cells transfected with SiNc(R)-NP (25 μg/mL, black curve) exposed to the laser diode (785 nm, 1.3 W/cm^2). Non-transfected cells exposed to the laser diode were used as the control (red curve). (**B**) Relative intracellular ROS level in A2780/AD cells incubated with SiNc(R) and SiNc(OH) complexes (25 μg/mL) and irradiated for 10 min using the 785 nm laser diode (1.3 W/cm^2).

2.4 Combinatorial Phototherapy

We revealed the superior efficacy of the combinatorial phototherapy mediated by SiNc(R)-based nanoplatform on ovarian carcinoma cells (A2780/AD) with more than a 99% cancer cell death (Fig. 5A, yellow bar), in comparison to PDT (50% cells death, cyan bar) and PTT (87% cell death, magenta bar) alone. Notably, the toxicity of the nanoplatform was insignificant under dark conditions (no laser irradiation), indicating safety and specificity of the combinatorial phototherapy (Fig. 5A, red and green bars). We have also confirmed anticancer efficacy of the combinatorial phototherapy in nude mice bearing a human ovarian cancer xenograft.

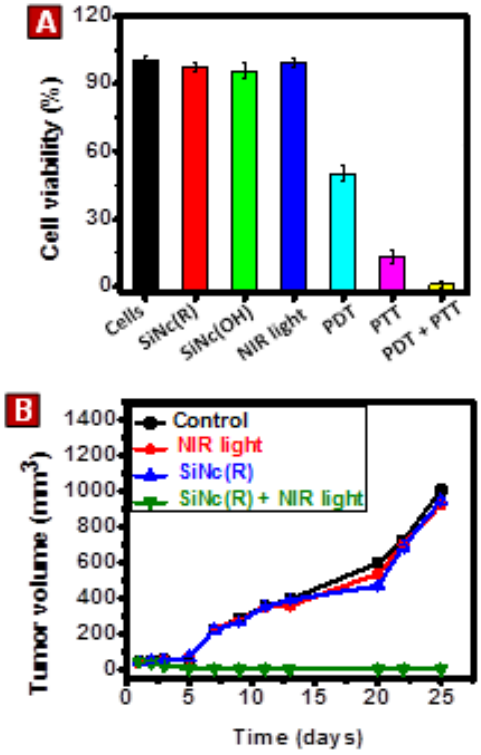

Figure 5: (**A**) Viability of DOX-resistant A2780/AD ovarian cancer cells after exposure to the following treatments: (1) no treatment (black bar); (2) SiNc(R)- and SiNc(OH)-based nanoplatforms under dark conditions (red and green bars); (3) NIR light (785 nm, 1.3 W/cm^2, 10 min, blue bar); (4) PDT: SiNc(R) platform + 785 nm NIR light (0.3 W/cm^2, 10 min, cyan bar); (5) PTT: SiNc(OH) platform + 785 nm NIR light (1.3 W/cm^2, 10 min, cyan bar); and (6) combinatorial phototherapy- SiNc(R) platform + 785 nm NIR light (1.3 W/cm^2, 10 min, cyan bar). (**B**) Growth of A2780/AD tumors in mice after treatment with: (1) saline (black); (2) 785 nm laser diode (1.3 W/cm^2, 10 min, red); (3) SiNc(R)-based nanoplatform (blue curve); and (4) combinatorial phototherapy: SiNc(R)-based platform combined with laser irradiation (1.3 W/cm^2, 10 min, green curve).

It is remarkable that solid tumors treated with a single dose of SiNc(R)-based nanoplatform combined with 10 min of NIR light irradiation (785 nm, 1.3 W/cm^2) shrank gradually and were completely eradicated from the mice with no evidence of cancer recurrence in the 25 day follow up period (Fig. 5B, green curve). Finally, not even slight skin burn was noticed after irradiating the non-treated tumors for 15 min, suggesting that 785 nm laser light at a power density 1.3 W/cm^2 is safe for phototherapy, making the application for the SiNc nanoplatform even more attractive. In vitro and in vivo studies revealed that combinatorial phototherapy mediated by SiNc(R)-based nanoplatform can efficiently destroy chemotherapy-resistant ovarian cancer cells and can potentially maximize the treatment effect of surgery by killing unresected cancer cells.

2.5 No need for Drug Release

Thus, the SiNc(R) nanoplatform can provide an efficient anticancer effect without releasing photoactive agent. The release of anticancer agents is one of the most important and limiting steps for drug delivery systems to exert efficient antitumor activity. Even if enhanced tumor accumulation of a drug carrier is accomplished, high antitumor efficacy cannot be achieved without reasonable drug release. The main advantage of the developed combinatorial phototherapy is that its anticancer efficacy is not limited by SiNc released from the dendrimer-based nanoplatform. To prove this point, the SiNc(R) release profile has been evaluated in PBS buffer at pH 5.5 containing 10 mM of reduced glutathione (GSH), mimicking intracellular (acidic and reduced) conditions.

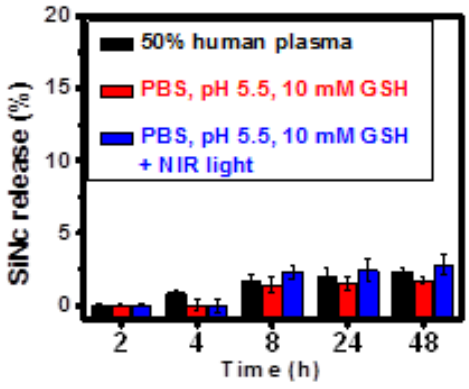

Figure 6: The release profile of SiNc(R) from dendrimer-based nanoplatform at 37 ºC in (1) 50% human serum; (2) PBS buffer at pH 5.5 with 10 mM GSH; and (3) PBS (pH 5.5, 10 mM GSH) + 785 nm light (1.3 W/cm^2, 20 min).

Our results, represented in Fig. 6 revealed that SiNc release was also minimal (less than 2%) under studied conditions. Taking into consideration the therapeutic efficacy studies (Fig. 5) and the SiNc release profiles (Fig. 6), one can conclude that the dendrimer encapsulated SiNc(R) molecules do not have to be released in the cancer cells to cause the PDT and PTT effects. Thus, due to the strong hydrophobic interactions between SiNc and the dendrimer interior, the release of SiNc is limited in aqueous setting, which prevents leaching of photoactive agents from the carriers in systemic circulation and, thus, minimizes side effects and maximizes delivery to the targeted cancer [10].

3 CONCLUSIONS

We reported a simple and effective approach for constructing a single SiNc-based theranostic agent for cancer imaging and treatment. The developed encapsulation strategy offers the possibility of separating the SiNc molecules thus decreasing their aggregation, preserving their NIR fluorescence signal, stabilizing their PDT and PTT properties and enhancing their water solubility. Furthermore, SiNc-NP exhibits minimal dark cytotoxicity and remarkable combinatorial phototherapeutic effects on ovarian cancer in vitro and in vivo, through its cytotoxic effects that cause a high production of ROS and heat without releasing the photoactive agent from the nanoplatform. Finally, the potential of SiNc-NP as an NIR imaging agent was confirmed by recording the strong fluorescence signal in the tumor area. We anticipate that the developed theranostic nanoplatform can be potentially applied for NIR fluorescence image-guided surgery and intraoperative cancer treatment.

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