Published in final edited form as:

Nano Lett. 2015 April 08; 15(4): 2249–2256. doi:10.1021/nl504044p.

Nanoscintillator-Mediated X-ray Inducible Photodynamic Therapy for In Vivo Cancer Treatment

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Abstract

Photodynamic therapy is a promising treatment method, but its applications are limited by the shallow penetration of visible light. Here, we report a novel X-ray inducible photodynamic therapy (X-PDT) approach that allows PDT to be regulated by X-rays. Upon X-ray irradiation, the integrated nanosystem, comprised of a core of a nanoscintillator and a mesoporous silica coating loaded with photosensitizers, converts X-ray photons to visible photons to activate the photosensitizers and cause efficient tumor shrinkage.

Graphical abstract

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ASSOCIATED CONTENT

Supporting Information

Supplementary figures and their legends, detailed materials and methods. This material is available free of charge via the Internet at <http://pubs.acs.org>

Notes

The authors declare no competing financial interest.

Keywords

nanomedicine; X-PDT; nanoscintillator; radioluminescence nanophosphors; singlet oxygen; cancer treatment

Photodynamic therapy (PDT) is a relatively new modality for cancer treatment.¹ PDT consists of three essential components: light, oxygen, and a phototosensitizer.^{1, 2} Photosensitizers, often pharmacologically inactive without illumination, can be activated by light of a specific wavelength. This activation is followed by transfer of energy to nearby oxygen molecules to generate cytotoxic reactive oxygen species (ROS), most importantly singlet oxygen ($^1\text{O}_2$).² PDT is a relatively less invasive treatment modality, inducing low systematic toxicity and causing little intrinsic or acquired resistance.^{1–3} One primary downside of PDT, however, is its inability to treat tumors located deep under the skin due to the short penetration depth of light in tissues.⁴ This problem can be partially alleviated by advanced light-delivering technologies that allow for illumination of certain internal cavities, such as the bladder, prostate, lung, and esophagus.^{5, 6} Nonetheless, it is considered challenging or impossible for conventional PDT to treat tumors of large volumes⁷ or multiple loci.⁸ Recently, there have been exciting developments of novel PDT derivatives, such as two-photon PDT or upconversion nanoparticle-mediated PDT, which aim to minimize tissue interference and improve penetration depth.^{1, 4, 9–11} However, given that the methods are powered by light, the treatment efficiency may still be highly surface-weighted.

We herein report a novel, $\text{SrAl}_2\text{O}_4:\text{Eu}^{2+}$ (hereafter referred to as SAO)-mediated X-ray inducible PDT (X-PDT) methodology, which as the name suggests regulates photosensitizer activation by X-rays. A similar concept was raised previously by the Chen group.^{12, 13} The rationale is that unlike visible or NIR light, X-ray photons have much greater penetration in body tissues. Should PDT be X-ray-activatable, the treatment could be initiated from virtually any part of a body with high efficiency.¹ Previously, the Chen group pioneered in demonstrating in vitro that X-ray can activate lanthanum fluoride or $\text{ZnS}:\text{Cu}, \text{Co}$ particles for cancer cell destruction.^{14, 15} Recently, the same group showed in vivo that copper-cysteamine microcomplexes of 5–20 μm can be used as sensitizers to produce $^1\text{O}_2$ under X-

ray irradiation.¹⁶ However, in these approaches, a 5 Gy X-ray irradiation was needed to achieve desired treatment effects, a dose that is comparable or even higher than the fraction doses used in clinical radiotherapy.^{17–19} Moreover, the fates of the scintillator materials in vivo and their long-term impact to the body are largely unknown.

In the present study, we address the issues with a novel integrated nanosystem, which consists of a core made of SAO and a silica coating loaded with a photosensitizer, merocyanine 540 (MC540) (Scheme 1). SAO is a strong luminescent material that can convert X-ray photons to visible photons, a phenomenon known as X-ray excited optical luminescence (XEOL).^{20, 21} Matching the excitation wavelength of MC540, the XEOL can activate MC540, producing cytotoxic ¹O₂. Impressively, we found in vitro and in vivo that a low X-ray dose (0.5 Gy, single dose) was sufficient to cause damage to cancer cells. In particular, we observed in murine U87MG xenograft models that SAO enabled X-PDT can induce prominent tumor growth arrest and even tumor shrinkage while leaving the normal tissues unaffected. Moreover, unlike the previously studied scintillator materials, SAO nanoparticles are highly hydrolytic. After the treatment, SAO nanoparticles are reduced to low-toxic ions and efficiently cleared from the host within 2 weeks, causing no long-term side effects. Overall, the SAO-mediated X-PDT inherits the benefits of PDT and potentially boasts excellent tissue penetration capacity. These properties suggest X-PDT as a promising treatment modality with great perspectives in the clinic, especially in oncology.

Synthesis, Surface Modification, and MC540 Loading of SAO Nanoparticles

Raw SAO was synthesized by carbothermal reaction using a vapor-phase deposition method.^{22, 23} Briefly, SrCO₃, Al₂O₃, Eu₂O₃, and graphite powders were mixed and heated in a tube furnace system at 1450 °C for 2 h under an argon flow with pressure maintained at 5 Torr. The as-synthesized SAO was wire-like (Figure 1a) and the main structure was identified to be monoclinic SrAl₂O₄ (JCPDS #74–0794) (Figure 1b).^{22, 23} Inductively coupled plasma mass spectrometry (ICP-MS) showed that Eu²⁺ accounts for 1% of the overall material mass. The material emits green photoluminescence and XEOL under UV and X-ray irradiation, respectively (Figure 1c,d). Both types of luminescence are attributable to the 4f⁶5d¹ → 4f⁷ transition of Eu²⁺ ions in the lattice.^{24, 25}

The bulkiness of the as-synthesized SAO makes it unsuitable for bioapplications. To reduce its dimensions, the SAO was mechanically ground, followed by sedimentation, filtration, and centrifugation, to yield ~80 nm (73.5 ± 26.9 nm) nanoparticles (Figure 2a). These SAO nanoparticles were then coated with a solid layer of silica, followed by further coating with an outer mesoporous layer (overall size 407.4 ± 152.5 nm, Figure 2b, Supporting Information Figure S1a,b,d,e). Each of the two silica layers plays a distinctive role in the nanosystem. The inner, solid silica coating functions as a protection shell that prevents contact between the SAO core and the surrounding aqueous environment (Supporting Information Figure S1a,b). This is essential because SAO is highly hydrolytic: Bare SAO nanoparticles are completely degraded in 5 min when directly exposed to aqueous solutions (Supporting Information Figure S1g). With the solid silica coating, the lifetime of SAO nanoparticles in aqueous solutions can be extended to more than 3 days (Figure 2c,d), which is sufficiently long for therapy purposes. The outer, mesoporous silica coating affords a

docking place for small molecules, a strategy commonly used in surface modification of nanoparticles.^{10, 26} Notably, for the mesoporous coating, we used both aminopropyltriethoxysilane (APTES) and tetraethyl orthosilicate (TEOS) as silane precursors. The resulting silica coated SAO nanoparticles (SAO@SiO₂ nanoparticles) have multiple amine groups on the surface and, thus, are slightly positively charged.

SAO@SiO₂ nanoparticles maintain the strong photoluminescence and XEOL of SAO. Figure 2e,f display emission spectra of SAO@SiO₂ nanoparticles under irradiation by UV/vis and X-ray light, respectively. Similar to the bulk material, both types of emission were found in the green spectral region, peaking around 520 nm (Figure 2e,f and Supporting Information Figure S2). The emission can be readily visualized on a Maestro small animal imaging system (Supporting Information Figure S3a–c), and even with the naked eye (Supporting Information Figure S3d,e).

Cellular Uptake and Cytotoxicity Studies

Cellular uptake was investigated with U87MG (human glioblastoma) cells. After incubation with SAO@SiO₂ nanoparticles (50 µg/mL) for 1 h, the cells were washed with PBS and imaged under a fluorescence microscope (Supporting Information Figure S4a). Green photoluminescence was observed in all the cells in the scope and was distributed across the cytoplasm but not in the nuclei.²⁷ This fits the pattern that SAO@SiO₂ nanoparticles were internalized by cells through endocytosis,^{28, 29} a process that may have been facilitated by electrostatic interactions between the particles and the cell membranes (Supporting Information Figure S4a).

Using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays, we then investigated the cytotoxicity of SAO@SiO₂ nanoparticles. We observed no significant viability drop even at high particle concentrations (up to 100 µg/mL, Supporting Information Figure S4b), suggesting good biocompatibility of our SAO@SiO₂ nanoparticles. However, MTT assays are only viable to assess short-term cytotoxicity (e.g., within 24 or 72 h) when most of the nanoparticles are still intact. As mentioned above, SAO is highly hydrolytic, and despite the presence of the SiO₂ coating, the nanoparticle core can be degraded in a physiological environment after 1 week (Figure 2c,d).^{30, 31} The released constituent ions, including Sr²⁺, Al(OH)₄[−] (the primary form at neutral pH),³² and Eu³⁺ (after oxidization of Eu²⁺), may affect cell viability differently. To investigate, in a separate study, we incubated bare SAO nanoparticles in water for 1 week to decompose SAO and then used the hydrolytes for toxicity assessments. There was again no significant drop of viability, confirming minimal toxicity of the nanoparticles even in the long run (Supporting Information Figure S5). This is not surprising since the major constituent ions of SAO have relatively low toxicity profiles. Sr²⁺ and Al³⁺ have been used in clinical medicines for applications such as postmenopausal osteoporosis, antacid, and bone implants.^{33–35} Eu³⁺ is a relatively nontoxic compared to other heavy metals^{36–38} and only accounts for 1% of the SAO nanoparticle mass.

$^1\text{O}_2$ Generation by X-PDT

The mesoporous coating allows easy loading of small molecules. Through overnight incubation, MC540 can be loaded onto SAO@SiO₂ nanoparticles. We chose a moderate loading rate of 15 wt % to minimize self-quenching among MC540 molecules. Despite the loading, the resulting MC540-loaded SAO@SiO₂ nanoparticles, or M-SAO@SiO₂ nanoparticles, remain highly stable in aqueous solutions (Supporting Information Figure S6).

There is a significant overlap between the XEOL of SAO and the excitation wavelength of MC540 (Figure 3a). Thus, it is hypothesized that when M-SAO@SiO₂ nanoparticles are irradiated by X-ray, SAO can relay energy in the form of visible photons to MC540 and as a result, produce $^1\text{O}_2$. To study the effect, we used a common $^1\text{O}_2$ indicator, singlet oxygen sensor green (SOSG).^{39, 40} SOSG is a fluorescent compound which undergoes a structural change in the presence of O₂, and the process is accompanied by an increase of fluorescence (ex/em: 504/525 nm). Hence, by measuring the fluorescence change, one can monitor $^1\text{O}_2$ generation in solutions or cells.^{39–41} Using SOSG, we first studied $^1\text{O}_2$ generation with a M-SAO@SiO₂ nanoparticle solution (50 µg/mL) under X-ray irradiation (1 Gy/h, Figure 3b). Compared to the background, the intensity of 525 nm fluorescence was increased by 8, 25, 35, 45, and 59% after 5, 10, 15, 20, and 30 min X-ray irradiation, respectively (Figure 3b). Meanwhile, no significant signal increase was observed during the intermissions of X-ray irradiations (Figure 3b). Similar studies were performed with solutions of MC-540, SAO nanoparticles, and PBS, all of which showed minimal increase of fluorescence under X-ray irradiation (Figure 3b). These data suggest that $^1\text{O}_2$ can and only can be produced when all the three components—MC-540, SAO, and X-ray—are present, corroborating our hypothesis that $^1\text{O}_2$ production is a result of SAO-mediated energy transfer.

The $^1\text{O}_2$ production was next examined with U87MG cells, again using SOSG as an indicator. For cells incubated with M-SAO@SiO₂ nanoparticles and irradiated by X-ray, there was a significant enhancement of 525 nm fluorescence. As a comparison, cells treated with nanoparticles only showed minimal fluorescence change (Figure 3c, Supporting Information Figure S7). By quantifying fluorescence readings from 10 images from each group (ImageJ, National Institutes of Health), it was determined that the fluorescence was enhanced by $410 \pm 29\%$ in cells treated by X-PDT (i.e., nanoparticle + X-ray). As with the observations made in the solutions, cells treated with M-SAO@SiO₂ only (without X-ray) or SAO@SiO₂ nanoparticles (with or without X-ray) showed only a marginal increase of fluorescence, again confirming that it takes the combination of X-ray, MC540, and SAO to generate $^1\text{O}_2$ (Figure 3c).

The produced $^1\text{O}_2$ translates to toxicity to cells. Figure 3d shows a cytotoxicity assay where ethidium homodimer-1 was used to mark dead cells (ex/em: 517/617 nm). Low red fluorescence intensity was observed with U87MG cells treated with X-rays alone (0.5 Gy) or with M-SAO@SiO₂ nanoparticles in the absence of X-ray irradiation (Figure 3d). In accordance with the $^1\text{O}_2$ generation results, the combination of M-SAO@SiO₂ nanoparticles and X-rays resulted in significant increase of red fluorescence within cells (Figure 3d, Supporting Information Figure S8 and Figure S9). The result was further

confirmed by MTT assays, which found a viability drop of $62.0 \pm 9.0\%$ with X-PDT-treated cells but little toxicity in all the controls (Figure 3e).

In Vivo Therapy Studies

An in vivo therapy study was conducted in murine subcutaneous tumor models. Briefly, 30 U87MG tumor-bearing mice were randomized to receive the following treatments ($n = 5$): (1) M-SAO@SiO₂ nanoparticles + X-ray, (2) M-SAO@SiO₂ nanoparticles only, (3) SAO@SiO₂ nanoparticles + X-ray, (4) M-SAO@SiO₂ nanoparticles only, (5) PBS + X-ray, and (6) PBS only. For Groups 1–4, SAO@SiO₂ or M-SAO@SiO₂ nanoparticles were intratumorally injected into the animals (2.5 mg M-SAO@SiO₂/mL, or 1.7 mg SAO/mL, 50 μ L of PBS for each mouse). For Groups 5 and 6, 50 μ L of PBS was intratumorally injected. For animals receiving X-rays (Group 1, 3, and 5), the irradiation was applied to the tumor area (1 Gy/h for 30 min, beam diameter 6 mm) 5 min after particle injection. Notably, this irradiation dose is far below those used in clinical radiotherapy (e.g., 60–80 Gy for solid epithelial tumors, 5 Gy per fraction).^{17–19}

Relative changes of tumor volume (V/V_0) are graphed in Figure 4a. For the treatment group (Group 1), tumor growth was immediately arrested after the treatment, followed by significant tumor shrinkage starting from day 6. On day 12, the average tumor volume was reduced to $60.2 \pm 6.9\%$ (Figure 4a). On day 16, three of the five animals showed almost impalpable tumors, leaving only thin scabs at the original tumor sites (Figure 4b and Supporting Information Figure S10 and Figure S11). All the animals in Group 1 were healthy throughout the whole study (Figure 4c). On the contrary, all the animals in the control groups showed rapid and comparable tumor growth (Figure 4a, Supporting Information Figure S10). On day 12, tumor volumes were increased by $768.0 \pm 87.0\%$, $797.4 \pm 98.6\%$, $776.9 \pm 91.9\%$, $767.4 \pm 80.8\%$, and $773.1 \pm 80.4\%$ for Groups 2–6, respectively (Figure 4a,b and Supporting Information Figure S13). By day 14, all the animals in the control groups had either died or been euthanized due to meeting at least one humane end point (Supporting Information Figure S12).

Postmortem H&E staining found densely packed neoplastic cells in tumors from the control groups (Figure 4d, Supporting Information Figure S13a). As a comparison, the treatment group showed drastically impacted tumor architectures and significantly reduced cell density (Figure 4d and Supporting Information Figure S13a), with many regions void of viable cells. Meanwhile, there was no detectable impact to the normal tissues, such as the heart, liver, spleen, kidneys, and skin (Supporting Information Figure S13b). This is attributed to the high selectivity of the X-PDT treatment (narrow and controllable beam irradiation) and also to the low toxicity and high biodegradability of SAO nanoparticles.

To further assess the excretion of SAO particles, in a separate study, we intravenously injected M-SAO@SiO₂ nanoparticles to normal balb/c mice. On day 16, we sacrificed the animals and evaluated the remaining Sr contents in different organs by ICP-MS analysis. For all the organs analyzed, we found that Sr contents were comparable to the background, confirming the efficient clearance of the particles (Figure 5a and Supporting Information

Figure S14). All of the injected animals were healthy throughout the whole study, with no weight loss (Figure 5b), skin toxicity, or any signs of morbidity.

Discussions and Conclusion

In the present study, the *in vivo* investigations were performed in subcutaneous tumor models. Owing to the excellent tissue penetration ability of X-rays, X-PDT has all the potential to treat tumors located deep under the skin. This was supported by a cytotoxicity study where U87MG cells were treated with M-SAO@SiO₂-mediated X-PDT, but with 4.5 cm thick pork positioned between the X-ray source and cells (Supporting Information Figure S15). Little difference in viability drop was observed relative to the cells receiving X-PDT but under direct X-ray exposure ($35.1 \pm 9.0\%$ vs $38.0 \pm 9.0\%$ for cells treated with and without pork, respectively, $P < 0.05$, Supporting Information Figure S16). This is in stark contrast to conventional PDT, which is not efficient when treating tumors located more than 1 cm under the skin (Supporting Information Figure S17).⁴²

A SAO-based nanoscintillator was used for X-ray-to-visible conversion, a step that is key to the X-PDT system. SAO is a well-known inorganic luminescent material and has been extensively used in areas such as safety indication, emergency lighting, road signs, billboards, graphic arts, interior decoration, lamp industry, radiation dosimetry, and color display.⁴³ In addition to its excellent optical properties, there are at least two more advantages of SAO for the current application. First, SAO forms an excellent energy pair with MC540, ensuring efficient intraparticle energy transfer that eventually leads to ¹O₂ production. Second, SAO is highly hydrolytic and its hydrolytes are relatively nontoxic.^{36, 37, 44} With silica as a semistable protection shell, the SAO core remains intact for a time span sufficient for the therapy and is then reduced to constituent ions that are readily excreted. This property minimizes long-term toxicity to the host, which is a common issue in nanoparticle-based imaging and therapy.¹¹

X-ray as an energy source is widely used in the clinic for both diagnosis and therapy purposes.^{20, 45} This suggests a minor hurdle from an instrument perspective as to the clinical translation of the technology. X-rays can be given to cover either a relatively small area (e.g., in external radiotherapy and dental radiography) or a large area (e.g., chest X-ray and CT). Both types of X-rays may be employed to power X-PDT. Although narrow-beam X-rays may induce more focal and selective damage, X-rays covering a large area may enable X-PDT to treat tumors of multiple loci or tumor metastasis. In the present study, M-SAO@SiO₂ nanoparticles were delivered directly to the cancerous sites, and treatment in this form resembles brachytherapy (although external X-ray irradiation is still needed as a trigger). This approach is expected to find wide applications in the clinic, especially for tumors that are resistant to radiation therapy. Meanwhile, it is possible to deliver nanoparticles systematically to tumors. For that purpose, a reduced nanoparticle dimension and optimized surface features are needed so as to minimize particle uptake by the reticuloendothelial system (RES) and maximize their accumulation in tumors.^{46, 47}

Future studies from different perspectives are needed to further improve the efficiency of X-PDT. First, optimize the particle core size, the coating thickness of each silica layer, and the

photosensitizer loading efficiency for maximized $^1\text{O}_2$ production. Because SAO particles are made from mechanical grinding, they show relatively wide size distribution. The issue can be compensated for in future with more advanced grinding technologies and improved size selection. Second, evaluate other scintillator materials of strong XEOL as transducers.²⁰ To this end, different photosensitizers with matching excitation wavelengths are needed, and the toxicity and biodegradability has to be reassessed. Third, investigate the impact of X-ray intensity and irradiation time on the treatment efficacy of X-PDT. This is important because the efficiency of PDT is often highly dependent on illumination fluence and fluence rate.¹

Overall, we have developed a SAO nanoscintillator-mediated X-PDT technology that allows efficient photosensitizer activation by low energy X-rays. The methodology inherits all the benefits of conventional PDT and allows for breach of the shallow penetration restraint, thereby opening many new possibilities. X-PDT as a novel and less invasive modality is expected to find wide applications in the battle against cancer and other diseases.

Method

In Vitro X-PDT

U87MG (human glioblastoma) cells (ATCC) were grown in DMEM medium supplemented with 10% FBS and 100 units/mL of penicillin (ATCC). The cells were maintained in a humidified, 5% carbon dioxide (CO_2) atmosphere at 37 °C. For viability studies, 10^4 U87MG cells were seeded in 96-well plates (Corning) and cultured for 24 h. The cells were then incubated with 50 $\mu\text{g/mL}$ M-SAO@ SiO_2 nanoparticles for 1 h. Subsequently, they were washed with PBS for two times, and then exposed to X-ray irradiation for 30 min (1 Gy/h). A mini-X X-ray tube (Amptek Inc.) was used as the X-ray source and was set at 50 kV and 70 μA for all the experiments in this study. Cell viability was evaluated by either dead assays (using ethidium homodimer-1 as a dead cell marker, Invitrogen) or MTT assays (Sigma-Aldrich) by following the vendor's protocols. For controls, cells were incubated with M-SAO@ SiO_2 nanoparticles but were not X-ray irradiated.

Animal Models

All the animal studies conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, U.S.A, and a protocol approved by the Institutional Animal Care and Use Committee (IACUC), University of Georgia. Animal models were established by subcutaneous injection of 10^6 U87MG onto the hind legs of 5–6 week athymic nude mice (Harlan).

In Vivo X-PDT

Therapy study began 3 weeks after tumor cell inoculation. Animals were randomized to receive the following treatments: (1) M-SAO@ SiO_2 nanoparticles + X-ray, (2) M-SAO@ SiO_2 nanoparticles only, (3) SAO@ SiO_2 nanoparticles + X-ray, (4) SAO@ SiO_2 nanoparticles, (5) PBS + X-ray, and (6) PBS only ($n = 5$). Nanoparticles were injected in 50 μL of PBS solutions to the tumors (2.5 mg M-SAO@ SiO_2/mL or 1.7 mg SAO/ mL , 50 μL of PBS for each mouse). For groups receiving X-ray irradiation, animals were irradiated by X-rays 5 min after the particle injection, at an irradiation dose of 0.5 Gy (over 30 min). Only

one therapy dose was applied to each animal. The tumor size and body weight of each animal were measured every other day. The tumor volume was calculated using the formula, tumor volume = length $\times$ (width)²/2. Tumors and major organs from the euthanized animals were harvested, weighed, and cryosectioned. The tissue sections were then subjected to standard H&E staining to assess treatment outcomes and side effects (BBC Biochemical).

Biodistribution Study

Normal balb/c mice (Harlan) were injected with M-SAO@SiO₂ nanoparticles (2.5 mg M-SAO@ SiO₂/mL or 1.7 mg SAO/mL, 50 μ L of PBS for each mouse, $n = 5$). The animals were euthanized 16 days after the injection, and the major organs, such as the liver, kidneys, heart, and spleen, were collected and weighed. The tissues were incubated in hot 70% nitric acid (Sigma-Aldrich) until they were decomposed and the solution became clear. The samples were centrifuged to remove remaining debris and the supernatants were analyzed by ICP-MS for strontium concentrations. The strontium contents in the organs were computed and expressed as nanograms/grams of tissue.

Statistical Analyses

In a therapy study, 30 tumor bearing mice were randomly divided into six groups. Two investigators were blinded when measuring tumor sizes and assessing treatment outcomes. Quantitative data were expressed as mean $\pm$ s.e.m. A two-tailed Student's t test was used for statistically comparing the treatment group and the control group. $P < 0.05$ was considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Wei Tang and Trevor Todd for their assistance on cell and animal studies and Sullins Benson for his contribution to graph design. The authors also acknowledge Dr. Xufan Li for his help on characterizations of SAO. This research was supported by an NCI/NIH R00 grant (5R00CA153772, J.X.), a UGA-GRU seed grant (J.X.), an NSF CAREER grant (CAREER DMR-0955908, Z.W.P.), and the intramural research program of the National Institute of Biomedical Imaging and Bioengineering (NIBIB, X.C.). The research is also supported by an Elsa U. Pardee Foundation Award (J.X.). We also thank the funding support by the National Basic Research Program of China (2015CB931800, B.S.), National Natural Science Foundation of China (81130028, 31210103913, B.S.), the Key Grant Project of Heilongjiang Province (GA12C302, B.S.), the Ph.D. Programs Foundation of Ministry of Education of China (201123071100203, B.S.), and the Key Laboratory of Molecular Imaging Foundation (College of Heilongjiang Province, B.S.).

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Figure 1. Structure, composition, and optical properties of raw SAO. (a) Scanning electron microscopy (SEM) image of as-synthesized SAO. (b) X-ray diffraction (XRD) analysis result. The main product is monoclinic SrAl_2O_4 (JCPDS #74-0794). (c) The raw SAO product under 365 nm UV irradiation. Strong green fluorescence was emitted from the material. The image was taken by a digital microscope. (d) Image of a single SAO wire struck by a narrow X-ray beam (the hit point was circled by red dashed lines). The resulting green emission was disseminated to the surroundings or along the wire.

Figure 2.

Morphology and optical properties of SAO@SiO₂ nanoparticles. (a) Transmission electron microscopy (TEM) image of bare SAO particles. (b) TEM image of SAO@SiO₂ nanoparticles. The silica coating consists of an inner solid layer and an outer mesoporous layer. (c) Stability of SAO@SiO₂ nanoparticles. SAO@SiO₂ nanoparticles were incubated in a simulated body fluid. The SAO cores were stable at the beginning but were then degraded gradually. After 14 days, only empty silica shells were observed under TEM. Scale bars, 100 nm. (d) Change of photoluminescence (PL) intensity (ex/em: 360 nm/520 nm), relative to the PL at the beginning of the incubation. Coincided with the TEM observations in c, there was a gradual drop of the PL intensity over time. The error bars represent $\pm$ s.e.m. of three independent experiments. (e) Photoluminescence spectra of SAO@SiO₂ nanoparticles, taken under excitation by different wavelengths of light (300–450 nm). Maximum emission was observed at $\sim$ 520 nm. (f) XEOL spectrum of SAO@SiO₂ nanoparticles. Emission also peaked at $\sim$ 520 nm.

Figure 3.

X-PDT induced $^1\text{O}_2$ production and cytotoxicity. (a) Good overlap exists between the XEOL of SAO (red) and the absorbance of MC540 (black). (b) Comparison of $^1\text{O}_2$ production, using SOSG as an indicator (ex/em: 504/525 nm). Increased levels of $^1\text{O}_2$ were only observed with M-SAO@SiO₂ nanoparticles under X-ray irradiation. Notably, there was a 1 min intermission after each 5 min X-ray irradiation cycle. The error bars represent $\pm$ s.e.m. of three independent experiments. (c) X-PDT induced $^1\text{O}_2$ generation in cells. Similar to observations in (b), enhanced fluorescence at 525 nm, which signals $^1\text{O}_2$ generation, was only observed when cells were treated with M-SAO@SiO₂ nanoparticles and in the presence of X-ray irradiation. Scale bars: 100 μm . (d) Cytotoxicity studies using ethidium homodimer-1 as a dead cell marker (a.k.a. dead assay). Correlated to the observations in (c), cytotoxicity was observed when M-SAO@SiO₂ nanoparticles and X-ray were used in

combination. Ex/em: 530 nm/635 nm. Scale bars, 50 μ m. (e) MTT assay results. Cell viability was significantly reduced when cells were treated with X-PDT (M-SAO@SiO₂ nanoparticles plus X-ray), and was minimally affected in other conditions. The error bars represent $\pm$ s.e.m. ($n = 4$ per group).

Figure 4.

X-PDT for in vivo tumor therapy. (a) Tumor growth curves (V/V_0 , $n = 5$). Significant tumor suppression and shrinkage was observed with animals injected with M-SAO@SiO₂ nanoparticles and irradiated by X-ray. In all the control groups, tumors grew rapidly and in a comparable pace. By day 14, all the animals in the control groups had either died or been euthanized for meeting at least one humane end point. The error bars represent $\pm$ s.e.m. * $P < 0.05$. (b) Photographs of representative tumors taken from Groups 1–6. (c) Body weight curves. No significant decrease of body weight was observed with X-PDT-treated animals. The error bars represent $\pm$ s.e.m. (d) H&E staining on tumor tissues taken from Groups 1–6. Compared to all the controls, where densely packed neoplastic cells were observed throughout the mass, tumors treated by X-PDT manifested drastically impacted tumor architectures and significantly reduced cell density. Scale bars, 100 μ m.

Figure 5.

Biodistribution and change of body weight after intravenous injection of M-SAO@SiO₂ nanoparticles. (a) Strontium (Sr) contents in different organs on day 16. The results were based on ICP analysis results on tissue samples. No difference in Sr contents was observed relative to control animals that had not been injected with M-SAO@SiO₂ nanoparticles. This observation suggests that the SAO had been mostly degraded and excreted by day 16. The error bars represent $\pm$ s.e.m. of three independent experiments. (b) Body weight changes. No significant difference in body weight was observed between normal animals and those injected with M-SAO@SiO₂ nanoparticles. The error bars represent $\pm$ s.e.m. ($n = 5$ mice per group).

Scheme 1. Schematic Illustration of the Working Mechanism of X-PDTa

^aA nanoscintillator core made of SAO is coated with two layers of silica, an inner solid layer and an outer mesoporous layer. Into the mesoporous silica layer, a photosensitizer, MC540, is loaded. Under X-ray irradiation, SAO converts X-rays to visible light photons (XEOL). The visible light photons, in turn, activate near-by MC540 molecules to produce cytotoxic $^1\text{O}_2$ that destroys nearby cancer cells. $^3\text{O}_2$ is the ground-state oxygen.