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Nanoparticle-Based Photosensitizers under CW Infrared Excitation

Yanyan Guo, Manoj Kumar, and Peng Zhang*

Laboratory of Nanomaterials Science, Department of Chemistry, New Mexico Tech, Socorro, New Mexico 87801

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Photodynamic therapy (PDT) has been gaining acceptance in recent years as a technique for cancer treatment.[1–3] PDT utilizes a photosensitizer, which works as a light-sensitive drug, to cause damage to the target cells and/or tissue locally upon the illumination of light with appropriate wavelengths.[4] It is generally accepted that the therapeutic effect of PDT is based on the interaction between the excited photosensitizer and the surrounding molecules, generating reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$). ROS can cause oxidative damage to biological substrates and ultimately lead to cell death. As a key component in effective and efficient PDT, the photosensitizers, also called PDT drugs, should ideally be (1) specific to the target; (2) highly effective in producing ROS when exposed to appropriate illumination; (3) excitable by a wavelength close to the near-infrared region (800 nm to 1 μm), where tissue penetration of the illumination is at a maximum. Regarding the last desired feature, single photons with infrared wavelengths are usually too weak energetically to generate ROS. Thus, multiphoton excitation would be needed for infrared light to be used as illumination source.[4–8] Here, we report the synthesis and characterization of a type of nanomaterial capable of generating $^1O_2$ under continuous wave infrared excitation, based on photon upconverting nanoparticles (PUNPs). The results demonstrate that such nanoparticles have great potential for becoming a new type of versatile PDT drugs for photodynamic therapy.

Photon upconverting materials convert lower-energy light to higher-energy light through excitation with multiple photons. They have been known and used for some time in a number of different applications.[9] Materials with photon upconverting properties, usually containing lanthanide ions, are much less common than those with down-converting properties. They typically have narrower absorption and line emission spectra, are not susceptible to photobleaching, and are chemically stable. Although they do not directly produce ROS, we utilize the fact that they adsorb infrared photons and emit visible ones to further excite the photosensitizing molecules, thus indirectly causing the photosensitizing molecules to generate $^1O_2$ under infrared excitation.

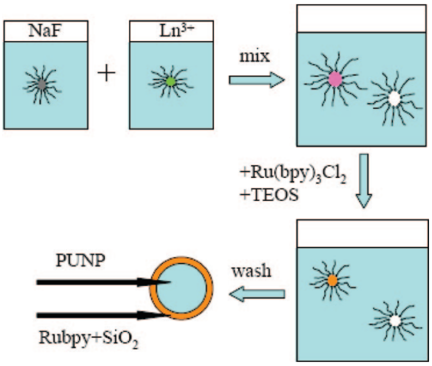

Figure 1. Schematic of PUNP-based photosensitizer.

The design and synthesis of the PUNP-based photosensitizers we proposed are schematically shown in Figure 1. The core was a $NaYF_4$:Yb^{3+},Tm^{3+} nanoparticle, a photon upconverting material capable of emitting blue light (~477 nm) upon excitation by an infrared light source (~975 nm). The nanoparticle was then coated by a thin layer of tris(bipyridine)ruthenium(II)-doped silica. $Ru(bpy)_3{}^{2+}$ (bpy = bipyridine) had long been known to generate $^1O_2$ with unit efficiency in a number of solvents when properly excited (absorbance maximum at ~450 nm).[10] Our hypothesis was that the embedded $Ru(bpy)_3{}^{2+}$ in the silica layer would adsorb the blue emission from PUNPs, and $^1O_2$ would then be produced in the reaction between the excited state of $Ru(bpy)_3{}^{2+}$ and triplet oxygen in solution.

The $NaYF_4$:Yb^{3+},Tm^{3+} nanoparticles were synthesized by a microemulsion method. The synthesis was carried out in a water/cetyl trimethylammonium bromide (CTAB)/hexanol microemulsion system. In brief, 0.2 M aqueous solutions of YCl_3, $YbCl_3$, and $TmCl_3$ and 0.415 M aqueous solutions of NaF were prepared and used as the water components in preparing two separate microemulsion solutions of the same H_2O/CTAB/hexanol ratio (16/24/60 by weight), one containing all lanthanide ions and one containing NaF. The two microemulsion solutions were mixed under rigorous stirring, and the reaction was allowed to proceed overnight.

The nanoparticles were then coated by a thin layer of $Ru(bpy)_3{}^{2+}$-doped silica through a variation of the well-known Stöber process. Calculated amounts of tetraethoxysilane (TEOS) and 1 mM $Ru(bpy)_3Cl_2$ aqueous solutions were added to the microemulsion solution under continuous stirring for 24 h at room temperature. The resulting nanoparticles were collected by adding acetone to break down the microemulsion system and washing the product a few times with methanol.

The final product was characterized spectroscopically and microscopically. The photoluminescence spectra of the

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* Corresponding author. E-mail: pzhang@nmt.edu.

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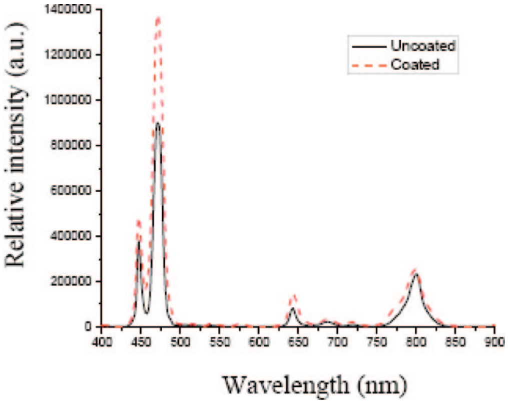

Figure 2. Photoluminescence spectra of $NaYF_4:Yb^{3+},Tm^{3+}$ nanoparticles before and after being coated with $Ru(bpy)_3^{2+}$-doped silica.

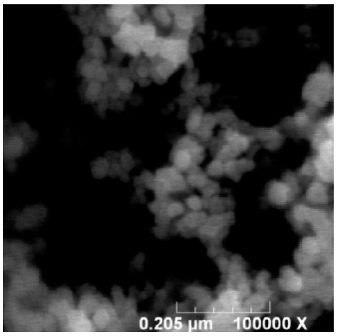

Figure 3. FESEM image of the final PUNP-based photosensitizers.

$NaYF_4:Yb^{3+},Tm^{3+}$ nanoparticles before and after being coated with $Ru(bpy)_3^{2+}$-doped silica, under 975 nm excitation, are shown in Figure 2. One can see that the coating does not affect the photon upconverting property of the $NaYF_4:Yb^{3+},Tm^{3+}$ nanoparticles. Figure 3is a FESEM image of the photon upconverting nanoparticles coated with $Ru(bpy)_3^{2+}$-doped silica. Most of the nanoparticles were in the range of 30−60 nm, with good monodispersity.

The generation of $^1O_2$ by these PUNPs-based photosensitizers was detected chemically, by using the disodium salt of 9,10-anthracenedipropionic acid (ADPA) as a probe molecule.[11] ADPA would be bleached by $^1O_2$ to its nonfluorescent endoperoxide. By measuring the decrease in the strong fluorescence emissions of ADPA, when excited at ~375 nm, one could monitor the generation of $^1O_2$.

Experimentally, 1 μL of 0.2 M ADPA solution was added to 3 mL of phosphate buffer solution (pH 7.2) containing 0.7 mg of nanoparticles, in each of three vials. The measurements were carried out under three sets of different conditions at different time intervals. The first vial was kept in the dark throughout the experiment, except for the light exposure when taking fluorescence measurements. The second vial was illuminated for 2 min by a 450 nm light from a 70 W xenon lamp through a monochromator before every fluorescence measurement was taken. The third vial was illuminated for 2 min by a 975 nm diode laser of 20 mW output before every fluorescence measurement was taken. All fluorescence measurements of ADPA were set at 420 nm emission/378 nm excitation.

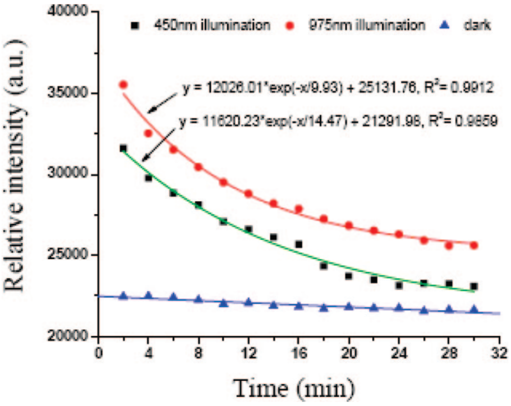

Figure 4. Change in intensity of ADPA emission at 420 nm, excited at 378 nm, for the respective solutions. The lines represent the fitting of the data points.

The results of the fluorescence measurements are shown in Figure 4. With no illumination, the ADPA fluorescence intensity barely decreased over time. When the solution was illuminated by 450 and 975 nm light sources, the fluorescence intensity decreased appreciably. Previous reports had shown that the intensity decrease in ADPA emission followed an exponential decay over time, as ADPA was being quenched by the generated $^1O_2$.[12–15] The data points in Figure 4for the two runs that were illuminated by 450 and 975 nm light, respectively, could also be fit into an exponential decay function, as indicated by the lines. This suggested that the kinetics of the $Ru(bpy)_3^{2+}$-coated PUNPs generating $^1O_2$ are very similar to those of other silica-based nanoparticles described in the literature.[12–14] The major difference was that the $Ru(bpy)_3^{2+}$-coated PUNPs were able to be excited by both IR (975 nm) and visible (450 nm) illuminations, whereas the others in the literature could be excited only by visible illumination. This difference would allow the penetration depth to increase several times for the PUNP-based photosensitizers, a highly desired feature in clinical applications.

We would like to point out the versatility of such PUNP-based photosensitizers. There are many Ru compounds, or other metal complexes, absorbing at ~470 nm and generating $^1O_2$ that can be readily used in this format. Furthermore, there are other PUNPs (such as $NaYF_4:Yb^{3+},Er^{3+}$ nanoparticles) available and/or being developed. They can also be adopted in this design, as long as appropriate compounds that absorb in the corresponding wavelengths and generate $^1O_2$ are included in the coating. We believe photosensitizers of such design hold great promise in photodynamic therapy.

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