Gold nanorods conjugated upconversion nanoparticles nanocomposites for simultaneous bioimaging, local temperature sensing and photothermal therapy of OML-1 oral cancer cells

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\textbf{ABSTRACT}

The major challenge in photothermal therapy (PTT) is to develop nanocomposites that simultaneously exhibit bioimaging and PTT under a single near-infrared (NIR) irradiation with high therapeutic efficiency. Herein, we present a multifunctional nanocomposite synthesized by linking NaYF\textsubscript{4}:Yb\textsuperscript{3+},Er\textsuperscript{3+} upconversion nanoparticles (UCNPs) with gold nanorods (AuNR) to exhibit fluorescence labeling, local temperature sensing and photothermal functions simultaneously with a single NIR laser excitation. The AuNR-NaYF\textsubscript{4}:Yb\textsuperscript{3+},Er\textsuperscript{3+} nanocomposite particles displayed better photothermal properties compared with pure AuNPs or a blend of AuNPs and NaYF\textsubscript{4}:Yb\textsuperscript{3+},Er\textsuperscript{3+} UCNPs. The temperature-dependent upconversion luminescence (UCL) property was used to determine local temperature at the nanocomposite particles, which is useful for selecting appropriate irradiation dosage for PTT. The therapeutic performance of the nanocomposites in PTT for OML-1 oral cancer cells was determined. For cell labeling, we successfully labeled streptavidin-linked nanocomposite particles on the surface of OML-1 oral cancer using anti-human epidermal growth factor receptor 2 (anti-Her2) antibody. Finally, the nanocomposite particles caused exceptional destruction of cancer cells up to 70% dead cells under 976 nm laser irradiation for only one min at 0.3 W/cm\textsuperscript{2} which is below the maximal permissible exposure of human skin.
1. Introduction

Currently, global cancer-causing deaths are estimated at 8.2 million cases per year and may reach 13 million by 2030 [1]. Along with medical advances, there have been a handful number of therapeutic methods discovered and developed. In addition to surgery, radiotherapy and chemotherapy, hyperthermia has been widely used as a treatment protocol, opening a new path in cancer therapy [2]. Recently, a variety of multifunctional nanocomposites have been developed for early cancer imaging and effective therapy [3–6]. They typically possess multimodal properties by combining two or more different elements into a single system for simultaneous imaging and hyperthermia therapy. Multimodal imaging-guidance integrated phototherapy offers more rapid, precise and reliable treatment for diseased cells [7]. Hybrid materials combining upconversion nanoparticles (UCNPs) and gold nanomaterials (AuNMs) have been proven promising for many biomedical applications [8–11], especially in bioimaging and photothermal therapy (PTT) [4–7].

For photoluminescence bioimaging probes, lanthanide-based UCNPs, capable of converting near-infrared (NIR) light into higher energy visible light, have been adopted for wide-ranging applications in versatile bioimaging and therapeutic tool [12–18]. The utilization of UCNPs can overcome some deleterious properties of traditional organic dyes as fluorescent labels, for example, photo-instability, biological incompatibility and photobleaching [19]. This is because UCNPs inherently possess unique invaluable features such as low photobleaching, long luminescence lifetime, high chemical stability, low

![UCL Imaging VIS](image-url)
cytotoxicity, biocompatibility, and ability to label particular cells [20,21]. Furthermore, they also have some additional advantages such as narrow emission band, large Stokes shift, NIR excitation leading to low-autofluorescence from biological samples, thereby substantially increasing the sensitivity and image contrast for biological imaging. With the rapid progress of nanotechnology, NaYF₄:Yb³⁺,Er³⁺ UCNPs have been shown to be one of the most efficient upconversion materials to generate strong upconversion luminescence (UCL) because NaYF₄ host lattices have low energy phonons (~350 cm⁻¹) and can accommodate a high concentration of lanthanide dopant ions, which are essential for facilitating UCL processes [22].

Nanomaterials used for PTT should possess high efficiency of converting absorbed light into localized heat energy, resulting in hyperthermia-induced cellular apoptosis or necroptosis [23]. Among various nanomaterials, such as metal nanomaterials [24–26], carbon nanotubes [27], graphene [28], and graphene oxide [29], gold nanorods (AuNRs) [30] have been considered as an ideal, robust, and versatile therapeutic nanoscale-agent due to their large and tunable localized surface plasmon resonance (LSPR). AuNRs can produce a rapid and efficient photothermal process because of their large absorption cross-section, resulting from LSPR [31], which is attributed to the strong collective oscillation of free electrons on the surface of AuNRs when they are illuminated with light at resonant wavelengths. Moreover, the aspect ratio and morphology of AuNRs can be easily controlled to tune their LSPR to the NIR region, overlapping with the biologically transparent window in the wavelength range from 750 nm to 1300 nm [32,33]. This permits deep penetration of light through biotissue and a minimal absorption by hemoglobin and water molecules in the human body [26]. It is noteworthy that AuNRs have been validated to possess the largest LSPR effect among all kinds of Au nanostructures, thus making them well-suited for photothermal applications [34]. For practical application of AuNRs in PTT, the temperature at AuNRs, defined as local temperature (LT), needs to be precisely monitored for the reduction of adverse side effects to healthy cells during the therapeutic process. In this context, lanthanide-doped UCNPs can serve as a nanothermometer to measure LT at AuNRs as long as UCNPs are conjugated with AuNRs to form AuNR-UCNPs nanocomposite particles. That is because the distance between AuNR and UCNPs is very short, and LT at UCNPs is close to LT at AuNRs. Consequently, real-time monitoring LT at AuNRs can be achieved by using AuNR-UCNPs nanocomposite particles. Together with UCL imaging and PTT functions, AuNR-UCNPs nanocomposite particles are well-suited to serve as a theranostic agent in cancer therapy.

Nevertheless, many previously reported multi-functional nanocomposites still face several drawbacks that limit their practical applications. They are: (i) the requirement of a complex dual-wavelength excitation system for simultaneous UCL imaging and PTT, due to non-overlapping of absorption bands of UCL imaging and PTT elements in nanocomposites systems [7]; (ii) the need for high-intensity laser irradiation, usually beyond the maximum permissible exposure of human skin (e.g., 0.4 W/cm²) [35] to perform PTT because AuNRs are excited through an indirect luminescence resonance energy transfer (LRET) process, which subsequently decreases photothermal conversion efficiency [4–6]. To overcome these two problems, herein we present a multifunctional hybrid AuNR@Silica-UCNPs nanocomposite based on the conjugation of NaYF₄:Yb³⁺,Er³⁺ UCNPs and silica-coated AuNRs with the same absorption peaks at 976 nm. The hybrid
nanocomposite particles can produce high efficient photothermal and UCL processes under the excitation of 976 nm NIR light with intensity lower than 0.4 W/cm². One crucial factor to promote the application of PTT in clinics is the penetration depth of laser irradiation into tissue. It is well known that NIR laser light can penetrate deeper in tissue in comparison with visible laser light because of less absorption and scattering [32,33,36,37]. To have a deeper penetration in tissue, we chose a 976 nm NIR laser, adapted with a fiber output coupler, as the excitation light source and the AuNR@Silica-UCNPs nanocomposite as PTT agent. Using the fiber optics output, it will be easier to deliver excitation laser light to tumor sites. Furthermore, oral cancer cells were chosen as the investigation target because most oral cancer tumors are close to the skin surface which do not require high penetration depth excitation. In addition, the advantages of using PTT for oral cancer treatment include: (i) the treatment process can be easily visualized and (ii) the general anesthesia is not required. In addition, streptavidin molecules are linked to the hybrid multi-functional nanocomposite particles, which can be indirectly bound to oral cancer cells via biotinylated secondary antibodies and anti-Her2 antibodies to detect human epidermal growth factor receptor 2 (Her2), a cancer marker overexpressed on the surface of oral cancer cells, through the assistance of the specific binding between streptavidin and biotin [38,39]. As a result, the AuNR@Silica-UCNPs nanocomposite particles are capable of labeling oral cancer cells effectively.

2. Materials and methods

2.1. Chemicals and materials

Oleic acid (OA) and 1-octadecene (ODE) (technical grade, 90%), yttrium (III) chloride (YCl₃, anhydrous powder, 99.99%), ytterbium (III) chloride (YbCl₃, anhydrous powder, 99.9%), erbium (III) chloride (ErCl₃, anhydrous powder, 99.9%), and ammonium fluoride (NH₄F, anhydrous powder, 99.99%) were purchased from Sigma-Aldrich and stored in a drybox. Sodium oleate (97%) was obtained from TCI America. MPTMS ((3-mercaptopropyl)trimethoxysilane, 97%) and TCAB (tetradecylammonium bromide, 98%) were purchased from Fluka. CTAB (hexadecyltrimethylammonium bromide, 99%), APTMS ((3-aminopropyl)trimethoxysilane, 98%), EDC (N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride), NHS (N-hydroxysuccinimide, ≥ 97%) and polyacrylic acid (PAA, Mw ≈ 1800) were obtained from Sigma-Aldrich. Sodium hydroxide (NaOH, pellet, 98%) was brought from Macron Fine Chemicals. Streptavidin protein was purchased from Rockland Immunocchemicals Inc and stored at 4°C. Anti-Her2 antibody and goat anti-rabbit IgG H&L (biotin) were purchased from Abcam and stored at −20°C.

2.2. Synthesis of hydrophobic UCNPs-OA

The monodisperse hexagonal-phase (β-phase) NaYF₄:Yb³⁺,Er³⁺ nanocrystals were synthesized through a thermal decomposition process as our previous study [40]. The typical procedure is as follows: 0.78 mmol of YCl₃, 0.2 mmol of YbCl₃, 0.02 mmol of ErCl₃ were mixed with 6 mL of OA and 15 mL of ODE into a 100 mL three-necked flask. The resulting solution was heated to 160°C with vigorous magnetic stirring under a nitrogen atmosphere until obtaining the pellucid suspension, which was then subjected to vacuum
and heated to 110°C for 1 h. After the heating, the reaction solution was allowed to cool down to 50°C, and then sodium oleate (0.76 g) was added. Finally, 10 mL of the methanol solution containing NH₄F (0.148 g) was slowly added to the solution and stirred for 30 min. Afterward, the solution was heated to 110°C to remove methanol until no obvious bubbles could be seen, then placed under vacuum and heated to 110°C for 1 h. Under a nitrogen atmosphere, the mixture was heated at 300°C for 45 min, then cooled rapidly by a strong stream of nitrogen gas blown at the outside of the flask following the exclusion of the flask from the heating mantle. Finally, UCNPs capped with oleic acid (UCNP-OA) from the suspension were collected by centrifugation at 6000 rpm for 10 min to precipitate the nanoparticles completely. The washing procedure using a mixture of cyclohexane and ethanol (1:1 v/v) was performed several times to ensure washing off the reaction surfactants as well as any NaF impurities that were created. The resultant nanocrystals were redispersed in cyclohexane for future use.

2.3. Synthesis of hydrophilic UCNP-PAA

The PAA-modified NaYF₄:Yb⁺,Er³⁺ UCNPs (UCNP-PAA) were prepared by using a modified ligand exchange strategy [40,41]. A typical procedure is as follows: the mixture containing 1 mL of PAA solution in ethanol (~1 wt%) and 0.5 mL of UCNP-OA dispersed in chloroform (~1 wt%) was stirred vigorously for at least 12 h at room temperature. The hydrophilic nanocrystals were then isolated via centrifugation at 7000 rpm for 30 min. After being further washed three times by absolute ethanol/deionized water (1:1 v/v) solution, the obtained PAA-capped UCNPs were finally redispersed in water to form a transparent colloidal solution without any obvious precipitation for more than four weeks.

2.4. Synthesis of AuNRs and silica-coated AuNRs

CTAB-coated AuNRs were prepared with the electrochemical method [42]. Next, the coating of a uniform thin silica shell on the surface of each AuNR was performed. A solution of 0.04 M NaOH was prepared in deionized water, and MPTMS was diluted in absolute ethanol, to form a 1% v/v MPTMS solution. The thickness of the silica shell of AuNRs surface could be controlled by varying the volume of the MPTMS solution. Concretely, a small amount (30, 40 or 50 μl) of MPTMS solution was added dropwise to the AuNRs suspension, and the mixture was stirred gently for 20 min. The silica coating with the sol-gel process on the AuNRs surface was performed by adding a fresh aqueous NaOH solution (pH ≈ 8.5) to the MPTMS-modified AuNRs suspension, also under slow stirring. Afterward, the mixture was allowed to react for 20 h before a 2:1 dilution with deionized water was made, to be used for subsequent measurements. Next, the surface of silica shells was further functionalized with amino group by APTMS. In a typical strategy, the washed silica-coated AuNRs were dispersed in 1 mL of ethanol. Then, 3 μL of APTMS was added to the suspension under shaking for 10 h. Finally, the suspension was centrifuged and washed four times with ethanol. After the last cycle, the obtained particles were redispersed in 1 mL of ethanol.
2.5. Preparation of AuNR@Silica-UCNPs

UCNP-PAA nanocrystals (0.04 mL, 5 mg/mL) were suspended in 0.5 mL of 10 mM MES (2-(N-morpholino) ethanesulfonic acid) buffer (pH = 5.5). Next, 100 μL of 1 mg/mL EDC and 50 μL of 1 mg/mL NHS were added to the suspension of UCNP-PAA and sonicated at 10°C for 1 h. Silica-coated AuNRs suspension (1 mL) was then added to the EDC/NHS activated UCNP-PAA colloids and sonicated for 1 h. The resulting silica-coated AuNR-UCNPs (AuNR@Silica-UCNPs) nanocomposite particles were then purified through centrifugation. Finally, the AuNR@Silica-UCNPs nanocomposite particles were dispersed in 1 mL of PBS (Phosphate-buffered saline) buffer (pH = 7.4).

2.6. Biofunctionalization of AuNR@Silica-UCNPs with Streptavidin

Streptavidin-functionalized AuNR@Silica-UCNPs hybrid nanocomposite particles were prepared by the crosslinking between the carboxylic acid groups on UCNPs and the amino groups on streptavidin. Typically, UCNP-PAA nanoparticles (0.04 mL, 5 mg/mL) were suspended in 0.5 mL of MES buffer containing 100 μL of EDC (1 mg/mL) and 50 μL of NHS (1 mg/mL) to activate carboxylic acid groups and sonicated at 10°C for 1 h. After centrifugation and washing with PBS buffer to remove the excess EDC/NHS, the precipitate was added to 1 mL of PBS buffer containing 300 μL of streptavidin solution (10^{-4} mg/mL) and silica-coated AuNRs. The linkage reaction was allowed to proceed at room temperature for 3 h. The streptavidin-functionalized nanocomposite particles (AuNR@Silica-UCNPs@SA) were isolated via centrifugation at 14,000 rpm for 30 min and then dispersed in PBS buffer (1 mL).

2.7. In vitro cytotoxicity assay

In vitro short-term cytotoxicity was tested on OML-1 oral cancer cells treated with the hybrid nanocomposites (AuNR@Silica-UCNPs). OML-1 cells were seeded in a 96 well-plate (1000 cells per well) and then cultured in an incubator under 37°C and 5% CO₂ for 24 h. Roswell park memorial institute (RPMI) 1640 medium mixed with 10% (wt/vol) fetal bovine serum (FBS) was used as the cell medium. After that, various concentrations of the nanocomposite particles (0, 2, 10, 50, 100μg/mL) were then added to the plates, followed by further incubation for another 48 h. The medium was then carefully removed, and 10 μL of cell counting kit-8 (CCK-8) solution (Sigma-Aldrich, St. Louis, MO) was added and incubated for another 4 h. The calculated cell viability was based on the absorbance measurement using a microplate reader (Multiskan FC) at a wavelength of 450 nm. The cell viability was analyzed by assuming 100% viability in the control cells (not treated with the hybrid nanocomposite particles).

2.8. In vitro UCL images

In vitro UCL images of OML-1 oral cancer cells were obtained by using a Nikon eclipse TE2000-U microscope adapted with an Infinity 3–3 URF CCD camera. The cancer cells were seeded for 24 h in a petri dish (2x10⁴ cells per dish) at 37°C in a 5% CO₂ humid atmosphere. Subsequently, the cells were washed with PBS for 3 times, fixed with 4%
paraformaldehyde for 30 min, then washed with PBS several times. Cells were then blocked with 5% (wt/vol) bovine serum albumin (BSA) in PBS containing 0.05% Tween 20 (PBST) for 30 min, followed by washing with PBS several times. For the detection of Her2 with AuNR@Silica-UCNPs@SA, both OML-1 oral cancer cells and immortalized ovarian surface epithelial (IOSE) cells were incubated sequentially with 2 µg/ml of anti-Her2 antibodies first for 30 min, and then 2 µg/ml of biotinylated goat anti-rabbit IgG H&L in PBST containing 5% BSA for 30 min. The anti-Her2 antibodies can conjugate to the OML-1 oral cancer cells surface. Next, the medium was discarded and fresh 5% BSA in PBST containing AuNR@Silica-UCNPs@SA (300 mL, 200 µg/mL) was added to each well and incubated at 4°C for 1 h. Subsequently, the cells were washed several times with PBST-BSA. Digital UCL images of cells were captured under NIR laser excitation, and the UCL with emission wavelength between 515 – 555 nm was detected by using a 535 nm bandpass filter.

2.9. In vitro photothermal therapy test

For the photothermal ablation test, a continuous-wavelength (CW) 976 nm wavelength NIR laser was used to expose the sample due to the large transmittance of biotissues at this wavelength and the overlap of the excitation laser wavelength with the longitudinal absorption band of AuNRs used in this work. Typically, the OML-1 oral cancer cells were loaded in a 96 well-plate (1000 cells per well), and approximately 100 µg/mL of hybrid nanocomposites (AuNR@Silica-UCNPs) were added for 4 h. After the treatment, the target cells were irradiated by a 976 nm NIR laser with 0.3 W/cm² intensity for 1 min. To examine cell viability, trypan blue was used to stain dead tissues or cells into blue by incubating with the cell dishes for 3 min. After staining, the cell images were examined using an optical microscope.

2.10. Characterization

The crystalline phase of UCNP-OA nanocrystals was characterized with a Bruker APEX diffractometer (λ = 1.5406 Å). The morphologies of the nanocomposite particles were characterized with a transmission electron microscope (TEM, JEOL, JEM-2010), and a high-resolution TEM (HRTEM, JEOL, JEM-2100). Meanwhile, the Fourier-transform infrared (FTIR) spectra of the as-prepared samples, which were dispersed in KBr pellets, were obtained using a Varian FTIR-640 spectrometer equipped with a liquid nitrogen-cooled mercur-y – cadmium – telluride (MCT) detector. The UV – Vis – NIR extinction spectra of AuNMs were obtained with full spectrum from 400 to 1100 nm by a Varian Cary 50 Bio spectrometer. The UCL spectra of samples were measured by using a grating spectrometer (Andor Shamrock SR-500i) under a 976 nm laser excitation.

3. Results and discussion

3.1. Characterization of the hybrid nanocomposites

The uniformity and morphology of UCNPs were examined by transmission electron microscopy (TEM). As indicated in Figure 1(a), the TEM image of NaYF₃:Yb²⁺,Er³⁺ UCNPs
clearly shows that those UCNPs have a spherical shape with a uniform size of approximately 10 ± 2 nm. The lattice fringes in the HRTEM image of a single NaYF₄:Yb³⁺,Er³⁺ UCNPs displayed in Figure 1(b) reveal that the as-prepared particles have a single-crystal nature. The (011) lattice planes of β-phase NaYF₄ crystal are clearly illustrated in the HRTEM image. The observed d-spacing of the (011) lattice planes is 0.29 nm [43], which is in good agreement with the X-ray diffraction (XRD) pattern. For the NaYF₄:20%Yb³⁺,2%Er³⁺ UCNPs, the powder XRD analysis reveals that the synthesized particles are with pure β-phase and no diffraction peak of the cubic-phase (α-phase) of NaYF₄ crystals and NaF impurities can be found (Figure 1(c)). It is well-known that β-phase NaYF₄ nanocrystals typically generate brighter UCL than α-phase NaYF₄ nanocrystals [18].

To prepare hydrophilic UCNPs, PAA was used to replace the original hydrophobic oleic acid on the surface of the NaYF₄:Yb³⁺,Er³⁺ UCNPs. Figure 1(d) shows the FTIR spectra of UCNP-OA and UCNP-PAA samples, which clearly displays the change of IR absorption before and after the surface modification. As shown in the FTIR spectra, the PAA-modified sample exhibits a broadband absorption at around 3500 cm⁻¹, which is assigned to the –OH stretching vibrations. For the UCNP-OA, the peaks at approximately 2927 and 2854 cm⁻¹ are attributed to the asymmetric and symmetric stretching vibrations of methylene (CH₂) in the long alkyl chain, respectively. The absorption peaks of 1559 and 1448 cm⁻¹ can be assigned to the asymmetric and symmetric stretching vibrations of the carboxylic group (COO–), respectively, indicating the presence of OA on the surface of the nanoparticles. After the surface modification with PAA, the peak of 2958 cm⁻¹ is assigned to the stretching and bending modes of CH₂, while the strong peak at 1717 cm⁻¹ corresponds to the C = O stretching vibration, and the two characteristic peaks centered at 1564 and 1405 cm⁻¹ are associated with the asymmetric and symmetric stretching vibration modes of the C-O group, respectively [44,45]. These observations suggest that PAA has been successfully immobilized onto the surface of the UCNPs. After the immobilization of the PAA ligand, the PAA-coated NaYF₄:Yb³⁺,Er³⁺ nanoparticles could be easily dispersed in water, forming a very stable colloidal solution without any noticeable precipitation over months. The presence of free carboxylic acid groups on the surfaces of UCNPs not only results in high solubility in water but also powerful chain for the UCNPs to link with AuNRs, by the strong coordination between the -COOH and the -NH₂ groups through the EDC/NHS coupling reaction.

The extinction spectra of AuNRs were taken immediately after completing the silica coating process and before the purification by quenching the sol-gel process with deionized water. As displayed in Figure S1(a), the LSPR longitudinal absorption band of AuNRs is red-shifted about 50 nm from 910 nm after the deposition of silica shell (AuNR@Silica). This is attributed to the change of refractive index on the surface of gold nanorods; i.e. from lower refractive index of water (1.33) to higher refractive index of silica (1.45) [46]. In addition, the width of the LSPR longitudinal absorption band remains unchanged after the coating of silica shell (see Figure S1(a)), which indicates that the silica-coated AuNRs are well dispersed in colloidal solution rather than agglomerated since aggregation will cause substantial broadening. Unlike CTAB stabilized AuNRs, which only disperse well in aqueous solution, AuNR@Silica can be easily dispersed in water, methanol, and ethanol. After APTMS functionalization, the absorption peak of the longitudinal LSPR of AuNR@Silica@APTMS sample is matched fairly well with the excitation wavelength (about 976 nm). To further confirm the presence of the amino groups onto
the silica surface after APTMS functionalization, the FTIR spectrum of the AuNR@Silica@APTMS sample was examined. As shown in Figure S1(b), the peaks of Si – O – Si and Si – OH bonds were observed at 1084 and 3454 cm\(^{-1}\), respectively. The peak associated with N – H stretching was detected at 1635 cm\(^{-1}\), clearly revealing the successful functionalization of the amino groups onto the silica surface in the surface modification process [47].

Figure S1(c) shows the surface zeta potential measurement results of the samples. The change of surface zeta potential, from negative (\(\zeta = -39.1\) mV) to positive (\(\zeta = 41.8\) mV) after the surface modification of AuNR@Silica with APTMS, is an evidence for the successful immobilization of amine-functional group on silica surface [48]. As displayed in Figure S1(c), the hydrophilic UCNPs-PAA exhibited a negative surface zeta potential (\(\zeta = -38.9\) mV) in neutral pH of deionized water [49], which suggests that the UCNPs-PAA can be conjugated with the amino group on the surface of AuNR@Silica@APTMS to form hybrid nanocomposites.

**Figure 2(a)** shows the UV – Vis – NIR extinction spectra of the colloidal solutions of silica-coated AuNMs, including gold nanospheres (NA530@Silica), short aspect ratio gold nanorods (NR650@Silica), and long aspect ratio gold nanorods (NR980@Silica), after surface functionalization with APTMS. The extinction spectrum of NA530@Silica contains a band around 530 nm, contributed by the LSPR of isolated gold nanospheres, and a shoulder located at 600 nm attributed to the aggregation of gold nanospheres [50]. There are two extinction peaks for both NR650@Silica and NR980@Silica: one is at 530 nm corresponding to the transverse LSPR and the other is associated with longitudinal LSPR located at 650 nm for NR650@Silica and at 980 nm for NR980@Silica. **Figure 2(b)** and (c), respectively, present the TEM images of NR650@Silica and NR980@Silica conjugated with NaYF\(_4\):Yb\(^{3+}\),Er\(^{3+}\) UCNPs, i.e. NR650@Silica-UCNPs and NR980@Silica-UCNPs nanocomposites. The average thickness of silica shells determined from TEM images of 100 particles, using SigmaScan Pro 5.0 image analysis software, is about 20 nm (see Figures S2(c-f)). We systematically investigated the effect of the LSPR wavelength of AuNMs on the UCL of UCNPs. These AuNMs were chosen due to the fact that their main LSPR wavelengths match with either the emission or excitation wavelength of the UCNPs.

### 3.2. Upconversion luminescence and photothermal properties of AuNM@Silica-UCNPs

**Figure 3(a)** shows the UCL intensities under 976 nm laser excitation at three major emission peaks of UCNP-PAA, NA530@Silica-UCNPs, NR650@Silica-UCNPs and NR980@Silica-UCNPs in water with the same concentration of UCNPs. Note that the UCL intensities of NA530@Silica-UCNPs, NR650@Silica-UCNPs and NR980@Silica-UCNPs nanocomposites were normalized with the corresponding UCL intensity of UCNP-PAA. The thickness of the silica coating of these nanocomposites is approximately 20 nm. Compared with the reference sample (UCNP-PAA), the UCL intensities at 525, 545 and 655 nm of NA530@Silica-UCNPs and NR650@Silica-UCNPs were enhanced about 1.1 to 1.2 times, due to the overlapping of the emission peaks of UCNPs and the LSPR bands of NA530@Silica and NR650@Silica (see **Figure 2(a)**). Meanwhile, for the case of NR980@Silica-UCNPs, its UCL intensities at the aforementioned wavelengths were much weaker than those of UCNPs-PAA. This is attributed to the high absorption of
NR980@Silica at the excitation wavelength of 976 nm. Most of the excitation energy was absorbed by AuNRs to induce photothermal effect and only a small amount of excitation energy was absorbed by UCNPs to produce UCL emission.

To investigate the photothermal properties of the NA530@Silica-UCNPs, NR650@Silica-UCNPs and NR980@Silica-UNCPs hybrid nanocomposites, we measured the temperature (T) of the colloidal solutions of these aforementioned nanocomposites as a function of the laser irradiation time with an intensity of 0.3 W/cm². In the measurement, a thermometer was immersed into the cuvette containing the colloids to determine the temperature of the colloidal solutions. The temperature was then recorded during the laser irradiation time from 0 to 12 min. Figure 3(b) plots the measurement results of the hybrid nanocomposites colloids as well as those of reference samples (UCNP-PAA colloid and water). As indicated in Figure 3(b), the NR980@Silica-UCNPs colloid had the most rapid and largest increase of temperature, \( \Delta T \approx 16^\circ C \) after 12 min of laser irradiation, thanks to the well-overlapped of LSPR longitudinal absorption band of the NR980@Silica and the excitation wavelength. It reveals that NR980@Silica-UCNPs nanocomposites possess very good photothermal property and are suitable for NIR excited PTT. The temperature increase in NA530@Silica-UCNPs, and NR650@Silica-UCNPs colloids were close to that of
UCNP-PAA colloid, ΔT ≈ 6°C after 12 min of laser irradiation. It indicates that NA530@Silica and NR650@Silica played an insignificant role in the rise of the temperature of the colloids. The increase in temperature was mainly contributed from UCNPs. Figure 3(b) also shows that water could absorb part of NIR excitation light and produced a small increase in temperature of water, ΔT ≈ 2.75°C after 12 min of laser irradiation.

To examine the effect of silica spacer layer thickness on the UCL and photothermal properties, the thickness of the silica spacer layer on the NR980 surface was tuned by varying the volume of MPTMS solution. As indicated in Figure S2, the silica thicknesses are determined to be 6.6 ± 1.4, 12.7 ± 1.2 and 19.3 ± 1.6 nm obtained by adding 30, 40 and 50 μL of MPTMS solution in AuNR colloids, respectively. From the TEM images of NR980@20nmSilica, the average width and length of the as-prepared NR980@20nmSilica are about 60.5 ± 1.7 and 152.1 ± 17.2 nm, respectively, determined by SigmaScan Pro 5.0 image analysis software (see Figure S3). Note that the nonuniform size and shape of Au nanorods appeared in Figure S2 may broaden the LSPR longitudinal band at 980 nm. However, its absorption is still much higher the absorption associated with the transverse band at 530 nm as revealed in Figure 2(a). The strong longitudinal LSPR absorption is expected to yield high efficient photothermal process under 976 nm excitation wavelength. Next, NaYF₄:Yb³⁺,Er³⁺ UCNPs with a diameter about 10 nm were conjugated onto the surface of silica spacer layer to form NR980@Silica-UCNPs nanocomposites with silica spacer layer thickness of 6, 12 and 20 nm. Figure 3(c) shows the UCL spectra of the aforementioned hybrid nanocomposites in water and there is no significant difference in UCL intensity with the change of silica spacer layer thickness. However, the UCL intensities of NR980@Silica-UCNPs hybrid nanocomposites were much weaker than that of UCNP-PAA because of the strong absorption of NR980@Silica at 976 nm excitation wavelength. Figure 3(d) plots the temperature of water versus the laser irradiation time of the aforementioned hybrid nanocomposites in water, which displays that the hybrid nanocomposites with 20 nm thick of silica spacer layer yielded the fastest and largest temperature increase in water, revealing that silica coating thickness may affect the photothermal property of the nanocomposites. This indicates that heat transfer is slower in thin silica-coated AuNRs than in the thick one. Our finding agrees with a result reported in [51], which indicates that the heat flux of a large nanoparticle is greater than that of a small counterpart. Therefore, at a constant excitation intensity, large nanoparticles, such as the hybrid nanocomposites with 20 nm thick of silica spacer layer, heat the surrounding environment more efficiently than small nanoparticles like hybrid nanocomposites with 12 or 6 nm thick of silica spacer layer.

### 3.3. The stability and photothermal conversion efficiency of nanocomposites

For practical applications in biological areas, an ideal therapeutic nanocomposite should be biochemical and physically stable. In order to examine the biochemical stability of the nanocomposite particles developed in this work, we dispersed NR980@20nmSilica-UCNPs nanocomposite particles into PBS buffer and culture medium (RPMI 1640 medium mixed with 10% (wt/vol) fetal bovine serum (FBS)). The TEM images of these nanocomposite particles were taken after different durations of incubation: 2 h, 6 h, 1 day and 3 days. The results indicate that the nanocomposite particles were stable in both PBS and culture medium within 1 day (see Figures S4(a-c), S5(a-c)). In contrast, the UCNP-PAA were
separated from the silica-coated AuNRs after 3 days of incubation (Figures S4(d) and S5 (d)). Therefore, the AuNR@Silica-UCNPs nanocomposite particles should be used within 1 day after preparation, as we did in this work.

We also investigated the fate of PAA coating after PTT treatment. In this investigation, two samples of UCNP capped with PAA were irradiated with 976 nm laser at 0.3 W/cm² intensity for 1 and 12 min, and one sample was heated at 65°C, i.e. the highest local temperature reached for the NR980@20nmSilica-UCNPs during PTT treatment (see section 3.4 below), for 1 min. The FTIR spectra of these samples were then taken to know the fate of PAA coating. As displayed in Figure S6, all the characteristic peaks of UCNP-PAA, centered at 3500, 2958, 1717, 1564 and 1405 cm⁻¹, still remained in these three samples. It reveals that the coating of PAA on the surface of UCNPs was thermally stable during PTT treatment at high temperature, which agrees with the previous finding [52].

The as-prepared AuNR@Silica-UCNPs nanocomposite particles exhibited very good photothermal property that inspired us to further study their photostability and photothermal conversion efficiency. Figure S7(a) shows the scheme of the experiment layout for the photostability and photothermal conversion efficiency measurement. The sample contained in a quartz cuvette was irradiated with a NIR CW diode laser at 976 nm. The laser beam was expanded with a pair of lenses and directed to the sample. A thermocouple connected with a digital thermometer was immersed into the solution to measure the temperature of the colloidal solution. In the measurement, an aqueous solution containing nanocomposite particles (NR980@20nmSilica-UCNPs) was irradiated by NIR 976 nm laser with 0.3 W/cm² intensity for 12 min, followed by naturally cooling to the original temperature without laser irradiation, which was one laser on/off cycle. The experiment was repeated for five laser on/off sequential cycles. The temperature change of the NR980@20nmSilica-UCNPs colloidal solution was recorded as a function of time. As displayed in Figure S7(b), in the first cycle, the temperature of the colloids increased from 19.4°C to 33.6°C by illuminating the laser beam for 12 min. After turning off the excitation source for 25 min, the temperature recovered to nearly its original value. After five cycles of irradiation, the temperature remained unchanged, indicating that as-synthesized nanocomposite particles have good photostability. This is beneficial for long-term clinical treatments using the AuNR@Silica-UCNP nanocomposite particles.

Figure 2. (a) UV – Vis – NIR extinction spectra of silica-coated NA530, NR650 and NR980 after surface functionalization with APTMS in water. TEM image of NaYF₄:Yb³⁺,Er³⁺ UCNPs conjugated onto the surface of silica-coated AuNRs: (b) NR650@Silica-UCNPs, (c) NR980@Silica-UCNPs.
Figure 3. (a) UCL emission intensities at 525, 545 and 655 nm of UCNP-PAA, NA530@Silica-UCNPs, NR650@Silica-UCNPs and NR980@Silica-UCNPs nanocomposites in water with the same concentration of UCNPs. (b) The temperature (T) of NR980@Silica-UCNPs, NR650@Silica-UCNPs, NA530@Silica-UCNPs and UCNP-PAA colloids as a function of the laser irradiation time. (c) UCL spectra of colloidal solutions of UCNP-PAA and NR980@Silica-UCNPs nanocomposites with silica spacer layer thickness of 6, 12 and 20 nm. (d) Temperature versus laser irradiation time in colloidal solutions of NR980@Silica-UCNPs nanocomposites with silica spacer layer thickness of 6, 12 and 20 nm.

The photothermal conversion efficiency ($\eta$) was calculated from the ratio of the energy received to the energy delivered, according to the following equation [53,54]:

$$\eta = \frac{hS(T_{\text{max}} - T_{\text{surr}}) - Q_{\text{dis}}}{I(1 - 10^{-A_{976}})} \quad (1)$$

where $T_{\text{max}}$ is maximum steady temperature, $T_{\text{surr}}$ is the environmental temperature of the solution, $Q_{\text{dis}}$ is the heat dissipated from light absorbed by container and solvent using a cuvette cell containing pure water without nanoparticles. $I$ is excitation intensity, $A_{976}$ is the absorbance of the NR980@20nmSilica-UCNPs nanocomposite particles at 976 nm wavelength. $h$ is the heat transfer coefficient, $S$ is the surface area of the container and the value of $hS$ was obtained from the following equations [53,54]:

$$\theta = \frac{T - T_{\text{surr}}}{T_{\text{max}} - T_{\text{surr}}} \quad (2)$$

$$t = -\tau_s \ln(\theta) \quad (3)$$
\[ hS = \frac{mC}{\tau_s} \]  

where \( \theta \) is a dimensionless parameter, \( C \) is heat capacity, \( m \) is the mass of the solution, \( \tau_s \) is the time constant for heat transfer and \( t \) is the time determined from the cooling period of the colloidal solution after laser irradiation.

From the cooling period in Figure S7(b), the decrease in the temperature of the colloids was further employed to determine the rate of the heat transfer from the NR980@20nmSilica-UCNPs nanocomposite particles to the surrounding environment. Figure S7(c) shows a plot representing \(-\ln(\theta)\) versus the cooling time during the cooling stage. The time constant for heat transfer \( \tau_s = 646.36 \text{ s} \) was determined from the slope of the fitting linear equation shown in Figure S7(c). The heat capacity \( (C) \) of the solution was assumed to be the same as the water of 4.2 J/g °C. The mass of the solution \( (m) \) was measured to be 1.0697 g. According to Eq. 4, the value of \( hS \) was then calculated to be 4.0 mW/°C. \( T_{\text{max}} \) and \( T_{\text{sur}} \) extracted from the data as shown in Figure S7(b) were 33.6 and 19.4°C, respectively. \( Q_{\text{dis}} \) was measured independently to be 52.2 mW using a quartz cuvette cell containing pure water. The incident laser intensity \( (I) \) was 0.3 W/cm² and the absorbance at 976 nm wavelength \( (A_{976}) \) of the used NR980@20nmSilica-UCNPs nanocomposites was 0.2. According to Eq. 1, the photothermal conversion efficiency \( (\eta) \) of the NR980@20nmSilica-UCNPs nanocomposite particles under 976 nm laser irradiation was determined to be 38.2%, indicating them highly superior as a promising PTT agent.

### 3.4. Sensing of the local temperature at hybrid nanocomposites

It is well-known that the electron populations of the \(^2\text{H}_{11/2}\) and \(^4\text{S}_{3/2}\) energy states of \( \text{Er}^{3+} \) ions follow the Boltzmann’s distribution in thermal equilibrium, and it leads to the intensity ratio of the \(^2\text{H}_{11/2}-^4\text{I}_{15/2}\) (UCL peak centered at 525 nm) and \(^4\text{S}_{3/2}-^4\text{I}_{15/2}\) (centered at 545 nm) transitions temperature-dependent. According to the Boltzmann’s distribution, the intensity ratio of these two green emissions can be expressed as a function of temperature [55–57]:

\[ \frac{I_{525}}{I_{545}} = C \times \exp \left( \frac{-\Delta E}{kT} \right) \]  

where \( I_{525} \) and \( I_{545} \) are the UCL intensities of the \(^2\text{H}_{11/2}-^4\text{I}_{15/2}\) and \(^4\text{S}_{3/2}-^4\text{I}_{15/2}\) transitions, respectively. \( C \) is a temperature-independent constant, \( \Delta E \) presents the energy gap between the energy levels \(^2\text{H}_{11/2}\) and \(^4\text{S}_{3/2}\), \( k \) is the Boltzmann constant, and \( T \) is the absolute temperature (in Kelvin scale).

The temperature-dependent UCL spectra of \( \text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+} \) UCNPs (UCNP-PAA) in water were first measured. In the measurement, a heater and thermometer were used to control and determine the temperature of colloid, respectively. Simultaneously, a laser at 976 nm with an intensity of 0.3 W/cm² was used to generate UCL emission which was then analyzed by a grating spectrometer. Figure 4(a) presents the emission spectra of UCNP-PAA in water at two different temperatures, 28°C and 85°C, under 976 nm excitation. This clearly demonstrates that the \( I_{525}/I_{545} \) intensity ratio increases with temperature as the re-distribution of electron population in the thermal equilibrium between the energy levels \(^2\text{H}_{11/2}\) and \(^4\text{S}_{3/2}\). Figure 4(b) shows a plot representing their logarithmic
intensity ratio \( R = \ln(I_{525}/I_{545}) \) as a function of the inverse absolute temperature (1/\( T \)). The experimental data is well-fitted to a linear equation:

\[
\ln(I_{525}/I_{545}) = 2.183 - 1128(1/\text{T})
\]  

\( (6) \)

The fitting equation was then used as a calibration curve to determine the local temperature (LT) at NR980@Silica-UCNPs nanocomposites.

In this study, the \( I_{525}/I_{545} \) intensity ratio of the UCL emitted from the NR980@Silica-UCNPs nanocomposites in water was first measured and then substituted into the Eq. 6 to calculate the LT at the nanocomposites. For comparison, the global temperature (GT) of the nanocomposites colloidal solution was measured by a thermometer immersed into the colloid. As shown in Figure 4(c) and S8, the \( I_{525}/I_{545} \) intensity ratio increases with the NIR laser excitation time. It represents that the LT at the nanocomposites increased with the laser exposure time. Figure S8 presents the UCL emission intensity ratio of the 525 and 655 nm emission bands as a function of the laser irradiation time. It is obvious that the \( I_{525}/I_{655} \) intensity ratio grows up with increasing the laser excitation time. The increase of the green-to-red intensity ratio derived from the reduction of the red emission intensity at high temperature is due to intensified lattice vibration, followed by enhanced non-
radiative relaxation process from $^4F_{9/2}$ energy state of Er$^{3+}$ ion [58]. Herein, we choose the $I_{525}/I_{545}$ intensity ratio to determine the LTs, because its absolute and relative sensitivities are larger compared to that of the $I_{525}/I_{655}$ intensity ratio [58]. Figure 4(d) plots the LTs and GTs of two types of hybrid nanocomposites (NR980@20nmSilica-UCNPs and NR980@12nmSilica-UCNPs) in water versus the NIR laser excitation time with 0.3 W/cm$^2$ intensity. It clearly shows that LTs were about 10°C to 20°C higher than GTs after 12 min of laser exposure. This is due to that LTs were measured at a nanoscale distance to the AuNRs excited by laser irradiation and they decreased rapidly from the surfaces of AuNRs. Whereas GTs were the average temperatures of the colloidal solution environment, which should be much lower than LTs. As illustrated in Figure 4(d), LTs rose rapidly at the early-stage of laser excitation, indicating the hybrid nanocomposites used in this work possessing high photothermal conversion efficiency. Especially, a faster and larger increase of LT was found in the hybrid nanocomposites with 20 nm thick of silica spacer layer. Therefore, they were chosen for the next step of PTT and UCL imaging investigations.

3.5. In vitro upconversion luminescence imaging

The determination of ErbB2 receptor tyrosine kinase 2 (ERBB2) or human epidermal growth factor receptor 2 (Her2) overexpression, a member of the epidermal growth factor (EGF) receptor family [38,59], in the cancer classification is an effective procedure. Anti-Her2 monoclonal antibody, the antibody directed to the extracellular domain of Her2 is used for the replacement or combinatorial therapy of Her2-overexpressing cancer cell. In addition, streptavidin (SA) has been widely employed in immunofluorescence labeling to improve in vitro targeting to tumor or cancer cell in the biotin-streptavidin labeling system. To evaluate the immunolabeling of the SA-conjugated nanocomposite probes, we incubated the probes (NR980@Silica-UCNPs@SA with 20 nm thick of silica layer) together with biotinylated goat anti-rabbit IgG and anti-Her2 antibody to OML-1 oral cancer cells. Figure 5(a) shows an overlay of the bright and dark field microscopy images (see Figure S9) of OML-1 cells after being incubated with the aforementioned materials. The bright UCL microscopy image of OML-1 cells confirms that the nanocomposite probes (NR980@Silica-UCNPs@SA) were effectively bounded onto the membrane of OML-1 oral cancer cells through the conjugation with

![Figure 5](image-url)

Figure 5. UCL microscopy imaging of OML-1 cells after incubating with streptavidin-conjugated nanocomposites (NR980@Silica-UCNPs@SA): OML-1 cells were treated (a) with anti-Her2 antibody, biotinylated goat anti-rabbit IgG, then incubated with streptavidin conjugates for 1 h, (b) without anti-Her2 and biotinylated goat anti-rabbit IgG antibodies, (c) IOSE cells were incubated with anti-Her2 antibody, biotinylated goat anti-rabbit IgG, then incubated with streptavidin conjugates for 1 h.
biotinylated goat anti-rabbit IgG and anti-Her2 antibody. The green luminescence is the UCL emitted from the NaYF₄:Yb⁺⁺,Er⁺⁺ UCNPs of the nanocomposite probes. The shape and position of the cells in bright field are overlapped well with dark field, showing the good specific interactions between the nanocomposite probes and the cells (see Figure S9(a,b)). In contrast, when the SA-conjugated nanocomposite probes were incubated alone (not incubated with anti-Her2 and biotinylated goat anti-rabbit IgG antibodies) with OML-1 cell or incubated with antibody (both anti-Her2 antibody and biotinylated goat anti-rabbit IgG) in immortalized ovarian surface epithelial (IOSE) cells [60] as shown in Figure 5(b) and (c), respectively, only weak green luminescence was observed on the cell surface. Furthermore, as indicated in Figure S10, no UCL signal was observed from the OML-1 cells incubated with NR980@Silica as well as the control cells (not incubated with the hybrid nanocomposites), because of the absence of UCNPs in both cases. These results prove that the hybrid nanocomposite probes with antibodies can bind with OML-1 cells. After examining superior UCL imaging, LT sensing and photothermal properties offered by the hybrid nanocomposite probes, it is worth to investigate the performance of the hybrid nanocomposites in PTT test.

### 3.6. Cell viability and photothermal therapy test

Initially, we evaluated the *in vitro* cytotoxicities of various nanoprobes, including NR980@Silica, NR980@Silica-UCNPs blending and NR980@Silica-UCNPs nanocomposites with silica average thickness of 20 nm, without NIR laser irradiation treatment by incubating different concentrations of nanoprobes (2, 10, 50, 100 µg/mL) with OML-1 cancer cells for 48 h. Then, the cell viability of oral cancer cells after the incubation of the aforementioned nanoprobes was investigated via a standard CCK-8 assay. As shown in Figure 6(b) (raw data shown in Tables S1-S4), the average cell viability remained above 90% after 48 h of the incubation of the nanoprobes, even at very high concentration (100 µg/mL). The result indicates that all the aforementioned nanoprobes have extremely low cytotoxicity, thus can be served as a reagent for oral cancer PTT.

Next, we examined the cancer therapeutic efficiencies of the aforementioned nanoprobes to OML-1 cancer cells via the exposure of a CW laser irradiation at 976 nm with an intensity of 0.3 W/cm² for 1 min. The exposure laser dosage can raise the LT of NR980@Silica-UCNPs to 42 – 45°C as shown in Figure 4(d). After laser treatment, the PTT effects of the test nanoprobes (100 µg/mL) were immediately determined by staining the OML-1 cancer cells with 100 µL of trypan blue for 3 min at room temperature. Without laser irradiation, only very small amounts of dead cells were found in the OML-1 cells incubated with and without (control sample) test nanoprobes, as indicated by the appearance of few points of trypan blue-stained cells. After laser irradiation, a remarkable difference was observed between the control sample and others. Incubating OML-1 cancer cells with the aforementioned nanoprobes resulted in efficient photothermal destruction of cells, and lots of blue color dots were clearly found (see Figure 6(a)). In comparison, no increase of blue color dots was found in the control sample, indicating most OML-1 cancer cells were survived after laser irradiation. As indicated in Figure 3(b), the temperature of the aqueous solution after 1 min of laser irradiation increased just 0.5°C. It is the reason why the laser exposure dosage had a negligible effect on the OML-1 oral cancer cells.
Figure 6. (a) Optical microscope images for PTT effects of samples of OML-1 cancer cells incubated with different nanoprobe before (-Laser) and after (+Laser) the irradiation of 976 nm laser with the intensity of 0.3 W/cm² for 1 min. Cell viability of OML-1 cancer cells (b) after incubation samples for 48 h without laser treatment and (c) after laser treatment for 1 min.

The cell viabilities of all the samples were quantitatively determined by a viable/dead cell CCK-8 assay. Figure 6(c) displays the cell viability of each sample before and after NIR laser irradiation (raw data shown in Table S5). After laser exposure, the control sample resulted in cell viability higher than 90%. It reveals that the laser irradiation dosage used in this work did not have any significant thermal effect on cells even though the 976 nm laser may lead to heat damaging effect to cells due to water absorption. On the other hand, the sample incubated with NR980@Silica-UCNPs nanocomposite particles had the lowest cell viability (30%), then the one with NR980@Silica-UCNPs blending (35%) and the one with pure NR980@Silica had about 50% of cell viability. This agrees with the result of GT change versus the laser irradiation time of these three samples as displayed in Figure S11, which confirms that the NR980@Silica-UCNPs nanocomposites possess the best photothermal conversion efficiency. That is attributed to part of the excitation energy absorbed by UCNPs was non-radiatively relaxed to thermal energy, which was easily coupled to the thermal energy produced by AuNR when UCNPs were conjugated to the surface of silica-coated AuNR. Hence, higher LT was achieved at the surface of the hybrid nanocomposites to yield higher cancer therapeutic efficiency. The conjugation of AuNR and UCNPs not only helps the integration of photothermal, UCL imaging and temperature sensing functions in the
hybrid nanocomposites but also greatly improves photothermal and cancer therapeutic efficiencies. Since the NIR laser intensity adopted in this work is much lower than those previously reported intensities [4–6] and is also slightly below the maximum permissible exposure intensity of human skin (0.4 W/cm²), the NR980@Silica-UCNPs hybrid nanocomposite particles are ideal to be served as an efficient photothermal agent in PTT of OML-1 oral cancer cells.

3.7. PTT performance comparison of different kinds of nanocomposites

Although different types of nanocomposites have been introduced, most of them still have drawbacks such as low photothermal conversion efficiency, the need for high dosage laser irradiation and a complex dual-wavelength excitation system for simultaneous bioimaging, local temperature sensing and PTT applications. There is still a lot of room for further improvement of PTT performance of nanocomposites. Table 1 summarizes PTT performance comparison of different kinds of nanocomposites. As indicated, the nanocomposites presented in Refs. [4–6] required excitation intensity higher than 0.6 W/cm² and long excitation time to perform PTT. Since the required excitation intensities were over the maximum permissible exposure of human skin [35], those aforementioned nanocomposites are impractical for PTT applications. In contrast, the nanocomposites presented in this work and Refs. [7,55] just needed low excitation intensity (0.3 W/cm²) and short excitation time to obtain high PTT efficiency thanks to their high photothermal conversion efficiencies. Thus, they are more suitable for PTT applications. Furthermore, the nanocomposite particles reported in this work required only a single NIR 976 nm laser source to perform bioimaging, local temperature sensing and PTT functions simultaneously, which is more promising compared to those complex dual-wavelength excited nanocomposites reported in Refs. [7,55].

SWE: single wavelength excitation for PTT and imaging
LTS: local temperature sensing
η: photothermal conversion efficiency
I_{exc}: excitation intensity
t: irradiation time
ξ: PTT efficiency of the nanocomposites at a concentration of 100 μg/mL
*: the concentration of nanocomposites is 200 μg/mL
N/A: not available

| Ref. | Nanocomposite | SWE | LTS | η (%) | I_{exc} (W/cm²) | t (min) | ξ (%) |
|------|---------------|-----|-----|-------|----------------|---------|-------|
| This work | AuNR@Silica-NaYF₄:Yb,Er | Yes | Yes | 38.2 | 0.3 | 1 | 70 |
| [3] AuNR@Silica-Quantum dots | No | No | N/A | 3.2 | 4 | N/A |
| [4] AuNR-NaGdF₄:Yb,Er | Yes | No | N/A | 1.2 | 10 | 50 |
| [5] AuNR-NaGdF₄:Yb,Er | Yes | No | 16.6 | 0.6 | 10 | 40 |
| [6] NaYF₄:Yb,Er@Silica-AuNR | Yes | No | N/A | 1.5 | 1 | N/A |
| [7] AuNR-dimer | No | No | 42.3 | 0.2 | 5 | *70 |
| [55] NaLuF₄:Yb,Er@NaLuF₄@Carbon | No | Yes | 38.1 | 0.3 | 5 | 55 |

Table 1. PTT performance comparison of different kinds of nanocomposites.
4. Conclusion

This work demonstrates the great potential of a multifunctional theranostic agent by the assembly of NaYF₄:Yb³⁺,Er³⁺ onto gold nanorods for simultaneous UCL bioimaging, LT sensing and PTT. The silica coating with a thickness of approximately 20 nm allows the AuNR to release more heat to its surrounding environment. We found that the local temperature of AuNRs reached to the suitable temperature (about 45°C) for the destruction of cancer cells after 1 min laser irradiation, which is higher than the global temperature of the surrounding environment thanks to the outstanding temperature sensing property of NaYF₄:Yb³⁺,Er³⁺ UCNPs. Afterward, the AuNR@Silica-UCNPs nanocomposites functionalized with anti-Her2 antibody can effectively label Her2 marker on the surface of OML-1 oral cancer cells. Moreover, the conjugation of AuNR and UCNPs yields high cancer therapeutic efficiency (about 70% of OML-1 cells were killed over one min treatment) using low-intensity irradiance at 976 nm wavelength. The results reveal a high potential therapeutic efficiency for targeting tumor via highly efficient photothermal effect combined with UCL labeling. We believe that AuNR@Silica-UCNPs nanocomposite particles developed in this study are feasible for multifunctional effective tool for UCL imaging, thermal sensing, temperature sensing and PTT applications to achieve a controllable cancer therapy.

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Disclosure statement

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References

[1] Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
[2] Wust P, Hildebrandt B, Sreenivasa G, et al. Hyperthermia in combined treatment of cancer. Lancet Oncol. 2002;3:487–497.
[3] Xia HX, Yang XQ, Song JT, et al. Folic acid-conjugated silica-coated gold nanorods and quantum dots for dual-modality CT and fluorescence imaging and photothermal therapy. J Mater Chem B. 2014;2:1945–1953.
[4] Song Y, Liu G, Dong X, et al. Au nanorod@NaGdF₄/Yb³⁺,Er³⁺ multifunctional hybrid nanocomposites with upconversion luminescence, magnetism, and photothermal property. J Phys Chem C. 2015;119:18527–18536.
[5] Wang C, Xu C, Xu L, et al. A novel core-shell structured upconversion nanorod as a multimodal bioimaging and photothermal ablation agent for cancer theranostics. J Mater Chem B. 2018;6:2597–2607.
[6] Chen CW, Lee PH, Chan YC, et al. Plasmon-induced hyperthermia: hybrid upconversion NaYF₄: “Yb/Er and gold nanomaterials for oral cancer photothermal therapy. J Mater Chem B. 2015;3:8293–8302.

[7] Sun M, Xu L, Ma W, et al. Hierarchical plasmonic nanorods and upconversion core-satellite nanoassemblies for multimodal imaging-guided combination phototherapy. Adv Mater. 2016;28:898–904.

[8] Rohani S, Quintanilla M, Tuccio S, et al. Enhanced luminescence, collective heating, and nanothermometry in an ensemble system composed of lanthanide-doped upconverting nanoparticles and gold nanorods. Adv Opt Mater. 2015;3:1606–1613.

[9] Zhang S, Wang J, Xu W, et al. Fluorescence resonance energy transfer between NaYF₄: “Yb,Tm upconversion nanoparticles and gold nanorods: near-infrared responsive biosensor for streptavidin. J Lumin. 2014;147:278–283.

[10] Chen H, Yuan F, Wang S, et al. Poly(acrylic acid) modification of Nd³⁺-sensitized upconversion nanophosphors for highly efficient UCL imaging and pH-responsive drug delivery. Adv Funct Mater. 2015;25:4717–4729.

[11] Nyk M, Kumar R, Ohulchansky TY, et al. High contrast in vitro and in vivo photoluminescence bioimaging using near infrared to near infrared up-conversion in Tm³⁺ and Yb³⁺ doped fluoride nanophosphors. Nano Lett. 2008;8:3834–3838.

[12] Yi Z, Lu W, Liu H, et al. High quality polycrylic acid modified multfunction luminescent nanorods for tri-modality bioimaging, in vivo long-lasting tracking and biodistribution. Nanoscale. 2015;7:542–550.

[13] Baziulyte-Paulaviciene D, Karabanovas V, Stasys M, et al. Synthesis and functionalization of NaGdF₄:Yb,Er@NaGdF₄ core-shell nanoparticles for possible application as multimodal contrast agents. Beilstein J Nanotechnol. 2017;8:1815–1824.

[14] Skripka A, Karabanovas V, Jarockyte G, et al. Decoupling theranostic with rare earth doped nanophosphors. Adv Funct Mater. 2018;29(12):1807105.

[15] Zhan Q, Qian J, Liang H, et al. Using 915 nm laser excited Tm³⁺/Er³⁺/Ho³⁺-doped NaYbF₄ upconversion nanoparticles for in vitro and deeper in vivo bioimaging without overheating irradiation. ACS Nano. 2011;5:3744–3757.

[16] Wen X, Wang B, Wu R, et al. Designed Er³⁺-singly doped NaYF₄ with double excitation bands for simultaneous deep macroscopic and microscopic upconverting bioimaging. Biomed Opt Express. 2016;7:2174–2185.

[17] Haase M, Schäfer H. Upconverting nanoparticles. Angew Chem Int Ed. 2011;50:5808–5829.

[18] Chen C, Li C, Shi Z. Current advances in lanthanide-doped upconversion nanostructure for detection and bioapplication. Adv Sci. 2016;3:1600029.

[19] Wang M, Mi CC, Wang WX, et al. Immunolabeling and NIR-excited fluorescent imaging of HeLa cells by using NaYF₄:Yb,Er upconversion nanoparticles. ACS Nano. 2009;3:1580–1586.

[20] Wang F, Liu X. Recent advances in the chemistry of lanthanide-doped upconversion nanocrystals. Chem Soc Rev. 2009;38:976–989.

[21] Liu Y, Xu M, Chen Q, et al. Gold nanorods/mesoporous silica-based nanocomposite as theranostic agents for targeting near-infrared imaging and photothermal therapy induced with laser. Int J Nanomedicine. 2015;10:4747–4761.

[22] Huang X, Jain PK, El-Sayed IH, et al. Plasmonic photothermal therapy (PTTT) using gold nanoparticles. Lasers Med Sci. 2008;23:217–228.

[23] Loo C, Lowery A, Halas N, et al. Immunotargeted nanoshells for integrated cancer imaging and therapy. Nano Lett. 2005;5:709–711.
[26] Mackey MA, Ali MRK, Austin LA, et al. The most effective gold nanorod size for plasmonic photothermal therapy: theory and in vitro experiments. J Phys Chem B. 2014;118:1319–1326.

[27] Kam NWS, O’Connell M, Wisdom JA, et al. Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. Proc Natl Acad Sci USA. 2005;102:11600–11605.

[28] Yang K, Zhang S, Zhang G, et al. Graphene in mice: ultrahigh in vivo tumor uptake and efficient photothermal therapy. Nano Lett. 2010;10:3318–3323.

[29] Yang K, Wan J, Zhang S, et al. The influence of surface chemistry and size of nanoscale graphene oxide on photothermal therapy of cancer using ultra-low laser power. Biomaterials. 2012;33:2206–2214.

[30] Huang X, El-Sayed IH, Qian W, et al. Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. J Am Chem Soc. 2006;128:2115–2120.

[31] Chen H, Shao L, Li Q, et al. Gold nanorods and their plasmonic properties. J Chem Soc Rev. 2013;42:2679–2724.

[32] Huang X, Neretina S, El-Sayed MA. Gold nanorods: from synthesis and properties to biological and biomedical applications. Adv Mater. 2009;21:4880–4910.

[33] Smith AM, Mancini MC, Nie S. Second window for in vivo imaging. Nat Nanotechnol. 2009;4:710–711.

[34] Hu M, Chen J, Li ZY, et al. Gold nanostructures: engineering their plasmonic properties for biomedical applications. Chem Soc Rev. 2006;35:1084–1094.

[35] Yuan H, Fales AM, Dinh TV. TAT peptide-functionalized gold nanostars: enhanced intracellular delivery and efficient NIR photothermal therapy using ultralow irradiance. J Am Chem Soc. 2012;134:11358–11361.

[36] Currà A, Gasbarrone R, Cardillo A, et al. Near-infrared spectroscopy as a tool for in vivo analysis of human muscles. Sci Rep. 2019;9:8623.

[37] Ash C, Dubec M, Donne K, et al. Effect of wavelength and beam width on penetration in light-tissue interaction using computational methods. Lasers Med Sci. 2017;32:1909–1918.

[38] Hou L, Shi D, Tu SM, et al. Oral cancer progression and c-erbB-2/neu proto-oncogene expression. Cancer Lett. 1992;65:215–220.

[39] Wu X, Liu H, Liu J, et al. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat Biotechnol. 2003;21:41–46.

[40] Vu DT, Chiu HW, Nababan R, et al. Enhancing upconversion luminescence emission of rare earth nanophosphors in aqueous solution with thousands fold enhancement factor by low refractive index resonant waveguide grating. ACS Photonics. 2018;5:3263–3271.

[41] Xia A, Deng Y, Shi H, et al. Polypeptide-functionalized NaYF<sub>4</sub>:Yb<sup>3+</sup>,Er<sup>3+</sup> nanoparticles: red-emission biomarkers for high quality bioimaging using a 915 nm laser. ACS Appl Mater Interfaces. 2014;6:18329–18336.

[42] Chang SS, Shih CW, Chen CD, et al. The shape transition of gold nanorods. Langmuir. 1999;15:701–709.

[43] Chen Y, Ai K, Liu Y, et al. Tailor-made-charge-conversational nanocomposite for pH-responsive drug delivery and cell imaging. ACS Appl Mater Interfaces. 2014;6:655–663.

[44] Ai X, Luu L, Mu J, et al. Synthesis of core-shell lanthanide-doped upconversion nanocrystals for cellular applications. J Vis Exp. 2017;129:1–9.

[45] Patel MM, Smart JD, Nevell TG, et al. Mucin/Poly(acrylic acid) interactions: a spectroscopic investigation of mucoadhesion. Biomacromolecules. 2003;4:1184–1190.

[46] Wu WC, Tracy JB. Large-scale silica overcoating of gold nanorods with tunable shell thickness. Chem Mater. 2015;27:2888–2894.

[47] Pasternack RM, Amy SR, Chabal YJ. Attachment of 3-(aminopropyl)triethoxysilane on silicon oxide surfaces: dependence on solution temperature. Langmuir. 2008;24:12963–12971.

[48] Liu F, Niu F, Peng N, et al. Synthesis, characterization, and application of Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub>–NH<sub>2</sub> nanoparticles. RSC Adv. 2015;5:18128–18136.

[49] Cheng L, Yang K, Li Y, et al. Facile preparation of multifunctional upconversion nanoprobes for multimodal imaging and dual-targeted photothermal therapy. Angew Chem Int Ed. 2011;50:7385–7390.
[50] Huang PJ, Chau LK, Yang TS, et al. Nanoaggregate-embedded beads as novel Raman labels for biodetection. Adv Funct Mater. 2009;19:242–248.

[51] Fedorenko SG, Romanishkin ID, Vanetsev AS, et al. Heating and cooling transients in the DyPO	extsubscript{4} nanocrystals under femtosecond laser irradiation in the NIR spectral range. Phys Wave Phenom. 2018;26:198–206.

[52] Mudhivarthi VK, Cole KS, Novak MJ, et al. Ultra-stable hemoglobin-poly(acrylic acid) conjugates. J Mater Chem. 2012;22:20423–20433.

[53] Liu X, Li B, Fu F, et al. Facile synthesis of biocompatible cysteine-coated CuS nanoparticles with high photothermal conversion efficiency for cancer therapy. Dalton Trans. 2014;43:11709–11715.

[54] Bi C, Chen J, Chen Y, et al. Realizing a record photothermal conversion efficiency of spiky gold nanoparticles in the second near-infrared window by structure-based rational design. Chem Mater. 2018;30:2709–2718.

[55] Zhu X, Feng W, Chang J, et al. Temperature-feedback upconversion nanocomposite for accurate photothermal therapy at facile temperature. Nat Commun. 2016;7:10437.

[56] Vetrone F, Naccache R, Zamarrón A, et al. Temperature sensing using fluorescent nanothermometers. ACS Nano. 2010;4:3254–3258.

[57] Nigoghossian K, Ouellet S, Plain J, et al. Upconversion nanoparticle-decorated gold nanoshells for near-infrared induced heating and thermometry. J Mater Chem B. 2017;5:7109–7117.

[58] Liu W, Wang X, Zhu Q, et al. Upconversion luminescence and favorable temperature sensing performance of eulytite-type Sr	extsubscript{3}Y(PO	extsubscript{4})	extsubscript{3}:Yb	extsuperscript{3+}/Ln	extsuperscript{3+} phosphors (Ln=Ho, Er, Tm). Sci Technol Adv Mater. 2019;20:949–963.

[59] Dokala A, Thakur SS. Extracellular region of epidermal growth factor receptor: a potential target for anti-EGFR drug discovery. Oncogene. 2017;36:2337–2344.

[60] Chan MWY, Huang YW, Frey CH, et al. Aberrant transforming growth factor β1 signaling and SMAD4 nuclear translocation confer epigenetic repression of ADAM19 in ovarian cancer. Neoplasia. 2008;10:908–919.