1. Introduction

Hyperthermia is a type of physical treatment for cancer therapy that has been widely used with chemotherapy to improve therapeutic efficiency and prevent drug resistance. To develop a high performance material for hyperthermia, the most important factors, such as effective therapy on the tumor region and efficient use of energy, should be considered. Traditionally, gold nanomaterials (Au NMs) such as nanoparticles (Au NPs), nanorods (Au NRs), and nanoshells (Au NSs), are most commonly used for hyperthermia; not only their enhanced permeability and retention effect could let them passively accumulate in the tumor environment, but their photothermal effect could also generate heat by direct laser irradiation. The photothermal effect is a strong non-radiative process caused by the surface of an electron that has a strong oscillation when Au NMs are irradiated with light. In this study, a near-infrared (NIR) laser is used as a treating source because it has a maximum permeability at physiological organization and a minimum affected quencher, such as water and hemoglobin in human body. To date, theranostic (i.e., a combination of therapeutic and diagnostic) has been the long-term goal in the development of personal nanomedicine; however, the Au NMs are difficult to apply directly in bio-imaging without further modification of other fluorescent dyes or other nanomaterials (NMs) and specific microscopy-like dark field and two-photon microscopy. Therefore, convenient multifunctional NMs are necessary for cancer treatment.

Plasmon-induced hyperthermia: hybrid upconversion NaYF₄:Yb/Er and gold nanomaterials for oral cancer photothermal therapy

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Nanocomposites consisting of upconversion nanoparticles (UCPs) and plasmonic materials have been widely explored for bio-imaging and cancer photothermal therapy (PTT). However, several challenges, including incomprehensible efficiency of energy transfer processes and optimization of the conditions for plasmon-induced photothermal effects, still exist. In this study, we fabricated NaYF₄:Yb/Er nanorods (Au NRs) conjugated with gold nanomaterials (Au NMs), such as Au NPs and gold nanorods (Au NRs). NaYF₄:Yb/Er NPs were used as photoconverters, which could emit green and red light under excitation of a 980 nm laser; Au NPs and Au NRs were also prepared and used as heat producers. The silica shell was further coated around UCPs to improve biocompatibility and as a bridge linking UCPs and the Au NMs. Most importantly, the thickness of the silica shell was tuned precisely to investigate the effective distance of the plasmonic field for heat induction. Energy transfer was confirmed by the declining UCL photoluminescence and emission decay time after connecting to the Au NMs. Moreover, a simulative model was built using the finite element method to assess the differences in heat generation between UCP@SiO₂-NPs and UCP@SiO₂-NRs. The surfaces of the hybrid nanocomposites were modified with folic acid to improve the specific targeting to cancer cells. The performance of the modified hybrid nanocomposites in PTT for OECM-1 oral cancer cells was evaluated.
In a method of combined photothermal therapy (PTT) and cell imaging, upconversion NPs (UCPs) are considered for use owing to their multi-emission in different regions, low excitation background, and long lifetime. UCPs have been developed for various applications, such as biosensors, water splitting, photodynamic therapy, and gene delivery because of their unique radiative process. UCPs consist of a host material (e.g., LaF$_3$, ZrO$_2$ and NaYF$_4$) doped with lanthanide ions (e.g., Yb$^{3+}$, Er$^{3+}$ and Ho$^{3+}$) as a sensitizer or an activator. Basically, lanthanide ions are the core factors in upconversion systems because of the two following reasons: (1) all lanthanide ions have a considerable number of 4f energy levels that are necessary for the upconversion process given that upconversion is a nonlinear process, which might absorb two or more photons and emit one photon with higher energy, such as UV, visible or NIR light. Emission color could also be tuned by doping different activators and the composition ratio of the sensitizer and the activator. Furthermore, Yb$^{3+}$, which is a commonly used sensitizer, has a large absorption cross-section at 980 nm. (2) The electron could easily transfer to neighboring levels because most energy levels are close with one another. However, the efficiency of the upconversion process is still a key problem because the nature of 4f–4f electronic transitions are Laporte forbidden. Therefore, many researchers have tried to enhance photoluminescence (PL) intensity through coating an inner or active shell with a similar crystalline material around the surface of UCPs to reduce the surface defect and interference by the solvent quencher from non-radiative decay. An active shell could further enhance the PL than an inner shell because the pumping photons will increase by co-doping a sensitizer in the core and the outer shell. UCPs coupled with plasmonic materials (e.g., Au NMs and Ag NMs) have also exhibited enhanced PL through surface-plasmon-coupled emission (SPCE) or the local field enhancement effect by increasing the emission rate or the excitation rate, respectively.

Moreover, plasmonic materials could be directly used as heat generators because of their photothermal effect. In hybrid UCPs and a plasmonic material system, the energy source for the PTT application could be an external laser or the UCPs. For the hybrid UCPs, Cheng and co-workers fabricated nanocomposites consisting of UCPs and iron oxide NPs which were covered with Au NS and then applied for cancer cell magnetic resonance imaging and PTT. The SPR absorption peak red-shifted after Au NP aggregation, which is similar to that for the Au NS. After the Au NS formation, the SPR peak slightly red-shifted, and another absorption band becomes obvious at the NIR region. Therefore, individual 980 and 808 nm lasers should be used for excitation in cancer cell upconversion luminescence (UCL) imaging and PTT, respectively. Moreover, Dong et al. fabricated NaYF$_4$:Yb$^{3+}$/Er$^{3+}$@Ag core/shell nanocomposites and used for PTT by exciting one 980 nm laser because Ag NS could tune the SPR peak to 980 nm. However, a fully coated outer shell formed by plasmonic NMs might cause unfavorable influence, in which not only the excitation light may be scattered, but also the UCP absorption efficiency may be decreased and the emission intensity may even be intensely quenched. For the plasmonic material system, heat is produced through luminescence resonance emission transfer (LRET) from UCPs to Au NMs. Qian et al. used a reverse micro-emulsion process to prepare hybrid nanocomposites, in which Au NPs are directly decorated on the surface of the silica shell. However, only a 4 °C difference was found between the heating performance of NaYF$_4$:Yb$^{3+}$/Er$^{3+}$@SiO$_2$-Au NPs and NaYF$_4$@SiO$_2$-Au NPs. Moreover, Song and co-workers constructed a unique Au NP@UCP core/shell structure, which has the potential for PTT application, but they only examined the temperature changes and confirmed that energy could be transferred to the Au core via a decrease in UCL emission. Both systems have no complete results to verify the optimized conditions for nanoplatform applications in cancer cell PTT with good performance, such as (1) confirmation of the energy transfer by other evidence, (2) the distance between UCPs and Au NMs that could exert high efficiency of the photothermal effect by the plasmonic field, and (3) the amount of Au NM loading that could give sufficient heat for PTT without quenching the UCL intensity.

In the present study, we fabricated multifunctional nanocomposites based on the conjugating upconversion of NaYF$_4$:Yb$^{3+}$/Er$^{3+}$ NPs and Au NPs or Au NRS through the combination of unique multi-emission and SPR effects for oral cancer PTT. We tried to evaluate which Au NMs have high potential of absorbing the energy from UCPs by the photothermal effect in terms of the difference in SPR absorption peak. UCPs were coated with a silica shell (with different thickness) to investigate the affected range of the plasmonic field, and Au NMs were directly decorated on the silica shell by electronic force. To improve the targeting ability, the silica shell was modified with folic acid (FA) by NHS/EDC coupling reaction. We also used a simulative model to study the dissimilitude between Au NPs and Au NRS with regard to the generation of heat under irradiation of the individual emission bands. The results showed that UCP@SiO$_2$-NR–FA has higher quality for heat production than UCP@SiO$_2$-NP–FA. The modified hybrid nanocomposites also exhibited an effective ability for cancer cell PTT and imaging.

2. Experimental
Chemicals and materials
Chloroauric acid, silver nitrate, citric acid, ascorbic acid, and CTAB were purchased from Acros. Ammonium solution (28–30%) was purchased from J. T. Baker. Y(CH$_3$CO$_2$)$_3$·xH$_2$O (99.9%), Yb(CH$_3$CO$_2$)$_3$·4H$_2$O (99.9%), Er(CH$_3$CO$_2$)$_3$·xH$_2$O (99.9%), NaOH (98%), NH$_4$F (98%), 1-octadecene (90%), OA (98%), and IGEPAL CO-520, TEOS (98%), APTES (98%), N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), chitosan (low molecular weight, 99%), monochloroacetic acid (≥99.0%), sodium borohydride (≥99.0%) and FA were purchased from Sigma-Aldrich. 4′,6-Diamidino-2-phenylindole (DAPI) was purchased from Invitrogen. Deionized water was obtained using a Milli-Q SP ultrapure-water purification system from Nihon Millipore Ltd, Tokyo. All chemicals were used as received without further purification.
Synthesis of NaYF₄:Yb³⁺/Er³⁺ NPs

NaYF₄:Yb,Er NPs were synthesized via a modified high-temperature co-precipitation method according to a literature procedure.²⁰ Y(CH₃CO₂)₁₂·xH₂O (85.13 mg; 0.32 mmol), Yb(CH₃CO₂)₃·4H₂O (25.21 mg; 0.072 mmol), and Er(CH₃CO₂)₃·xH₂O (5.51 mg; 0.008 mmol) were added into a 50 mL two-neck round-bottom flask with OA (3 mL) and 1-octadecene (7 mL). The solution was heated to 150 °C under the protection of nitrogen for 30 min to dissolve the lanthanide salts and cooled down to 50 °C. Subsequently, a methanol solution (3.0 mL) containing NH₄F (59.26 g; 1.6 mmol) and NaOH (0.04 g; 1.0 mmol) was added and stirred for 30 min. The reaction mixture was then heated at 100 °C for 30 min to remove the methanol; the solution was then heated to 290 °C and kept for 2 h before cooling to room temperature. The as-prepared NPs were precipitated by adding absolute ethanol, collected by centrifugation at 6000 rpm for 10 min, and washed with ethanol for several times. The UCPs were stored in cyclohexane for the next step.

Synthesis of UCP@SiO₂–NH₂

Silica shells were coated on the UCPs and further functionalized with amino groups through a reverse micro-emulsion.²⁰ Approximately 1 mL of CO-520 was dispersed in 10 mL of cyclohexane for 30 min stirring. Afterwards, approximately 10 mg of UCPs were prepared in 1 mL of cyclohexane solution and added into the mixture for 1 h. Approximately 0.1 mL of ammonium solution was added for another 1 h. Afterwards, different amounts of TEOS were slowly added using a syringe pump to control the flow rate in 400 μL h⁻¹, and the reaction was gently stirred for 24 h. The thicknesses of the silica shell to be 1.2, 2.2, 4.3, 7.9 and 12.9 nm could be tuned by adding 10, 20, 40, 80 and 100 μL, respectively. To graft amino groups on the surface, approximately 0.1 mL APTEs was slowly injected into the system, and kept under stirring for another 4 h. The product could be precipitated by adding methanol and collected by centrifugation (7800 rpm, 20 min). After washing several times by MeOH, UCP@SiO₂–NH₂ was finally dispersed in deionized water or EtOH.

Synthesis of UCP@SiO₂-NP–FA and UCP@SiO₂-NR–FA

The 3.5 nm Au NPs were synthesized using a typical method that used NaBH₄ as a strong reduction agent and citric acid as a capping ligand; Au NRs were prepared, and the SPR absorption peak of Au NRs were adjusted to match the UCL emission via a modified seed-mediated growth method.³¹ FA was then modified on the UCP@SiO₂ through NHS/EDC coupling reaction.¹² Briefly, FA, NHS and EDC were mixed for activation in a molar ratio of 1 : 2.5 : 1 (1.2, 3 and 1.2 μmol) for 1 h at RT. Afterwards, approximately 10 μg of UCPs@SiO₂–NH₂ were added to the mixture solution to react for another 12 h at RT. UCPs@SiO₂–FA was purified with EtOH by centrifugation (12000 rpm, 12 min). The obtained product UCP@SiO₂–FA could be dispersed into water or buffer solution for a long time with high stability. Before Au NPs and Au NRs were decorated on the UCP@SiO₂–FA, both Au NPs and Au NRs exchanged the surface ligand to OCMCS. This process was directly added with 0.5 mg mL⁻¹ OCMCS in the NP solution and stirred for at least 12 h. The CTAB concentration in Au NR solution should be washed by water until lower than 500 μM because the long chain from CTAB discouraged the ligand exchange reaction. UCP@SiO₂-NP–FA or UCP@SiO₂-NR–FA were then obtained from different volumes of Au NPs or Au NRs reacting with UCP@SiO₂–FA for 1 h. The products were then purified through centrifugation (10000 or 5000 rpm for the NP or NR system, respectively).

Photothermal ability of UCP@SiO₂-NP or UCP@SiO₂-NR in solution

To test the photothermal conversion for nanocomposites, approximately 200 μL of UCP@SiO₂-NP or UCP@SiO₂-NR (1 mg mL⁻¹ for each sample) were prepared in water, and the samples were placed in the quartz cuvette by directly irradiating 1.5 W cm⁻² at 980 nm laser for 3 min. The variation in temperature will be recorded using a thermocouple every 10 s.

Simulative analysis. All the simulated spectra and field distributions were obtained by solving three-dimensional Maxwell equations with FEM. The permittivity of gold is described by the Drude–Lorentz model with a plasmon frequency of ωp = 8.997 eV and a damping constant of Γp = 0.07 eV. The refractive index of water, UCPs, and SiO₂ is fixed at 1.33, 1.8 and 1.46, respectively. An electric dipole source with a current amplitude of 1 A m⁻¹ (where δ = 1/√3 ̂i + 1/√3 ̂j + 1/√3 ̂k) (where ̂i, ̂j, and ̂k are the unit vectors along the x-, y- and z-axis, respectively) is used to simulate the light emitted from UCPs. The scattering boundary condition is used to avoid the reflection influence. According to the UCP experimental spectra, three peak wavelengths (525, 540 and 655 nm) were employed for the induced heat study. The total number of photons emitted from UCPs was realized by measuring the laser power and the quantum yield of UCPs. According to the emission spectra, the power of the emitted light from UCPs in 1 A m⁻¹ (δ = 525, 540 and 655 nm) is 17, 67 and 21 W m⁻², respectively. The time average resistive heating Qave can be obtained by calculating the time average resistive heating,³¹ which is defined as follows:

\[ Q_{ave} = \frac{1}{2} Re(\sigma\vec{E}\cdot\vec{\nabla}\times\vec{E}) \]

where \( \sigma \) is the electric field, \( D \) is the electric displacement field, and \( \omega \) is the angular frequency. The induced heat is subsequently calculated by integrating the resistive heating on gold particles.

Cell viability assay and cellular uptake. The cell viability assay was applied on OECM-1 oral cancer cells treated with UCP@SiO₂, UCP@SiO₂-NP–FA, and UCP@SiO₂-NR–FA for 24 and 72 h. OECM-1 cell lines were cultured in an incubator under 37 °C and 5% CO₂ using the typical method, and the cell medium used was RPMI-1640 medium mixed with 10% fetal bovine serum and penicillin/streptomycin 10% glutamine. The concentration of nanocomposites (1, 3, 9, 27, 81 and 250 μg mL⁻¹) were then added to the plates and incubated for 24 h. For the 72 h
treatment, the cell number will also use 2000 mL−1 cells to prevent cell overgrowth, which can lead to dead or unhealthy cells. The resulting cell viability was calculated via an Alamar Blue assay using 560 EX nm/590 EM nm filter settings to determine the fluorescence intensity.

In vitro confocal images were obtained using a Leica TCS SP5 confocal microscope. Oral cancer cells (OECM-1) were incubated with UCP@SiO2, UCP@SiO2-NP–FA, and UCP@SiO2-NR–FA for 12 h in 6-well plates at 37 °C in a 5% CO2 humidity atmosphere. All of the cells were incubated on the top of the cover glass. The samples on the slides for capturing confocal images were prepared in several steps. Briefly, the culture medium was vacuumed to remove the free nanocomposites. Exponentially growing cells were fixed on the piece of cover glass using 4% paraformaldehyde, 1 mL per well for 5 min. The nuclei in the cultures were stained with DAPI for further 5 min to label the cells. After the staining procedure, the pieces of the cover glasses were stuck on the top of the slides with moderate mounting oil between them. A nuclei image was obtained by exciting an ultraviolet laser at 360 nm, and the emission was detected between 450–470 nm; UCP images were excited in an NIR laser at 980 nm, and the emission was detected between 500–560 nm.

PTT test. In the photothermal ablation experiment, the OECM-1 oral cancer cells were loaded in 12-well plates, and approximately 100 μg mL−1 were treated with a low and high Au/Y ratio of UCP@SiO2-NP–FA and UCP@SiO2-NR–FA for 12 h (UCP@SiO2, Au NPs and Au NRs as the control group, and both Au NMs of the concentrations were 100 mg mL−1). After the treatment, the nanocomposites were removed by PBS buffer (pH 7.4). Following incubation for 12 h in an incubator, the target cells were irradiated with 1.5 W cm−2 by a NIR laser at a 980 nm wavelength for 1 min. Trypan blue, a dye that is commonly used to color dead tissues or cells blue selectively, was added to the cell dishes for 30 min.

Characterization. The morphology of the samples was studied using TEM, with an electron gun operating at 100 keV. The TEM and HRTEM images were collected using a JEOL JEM-2100F electron microscope, and the crystalline phase of UCPs was characterized by 1.54 Å XRD pattern (Bruker D2 Phaser diffractometer). The UCL emission spectra of samples were obtained using a Varian FTIR-640 spectrometer. The gold concentration was measured using ICP-MS. The sample was investigated using confocal microscopy (Leica TCS SP2 AOBS) for the internalization efficiency of the NMs and the PTT test. UC PL lifetimes were measured using a customized UV to mid-infrared steady-state and phosphorescence lifetime spectrometer (FSP920C, Edinburgh Instrument) equipped with a digital oscilloscope (TDS3052B, Tektronix) and a tunable mid-band optical parametric oscillator pulse laser as the excitation source (410–2400 nm, 10 Hz, pulse width ~5 ns, Vibrant 355H, OPOTEK).

3. Results and discussion

Characterization of UCP@SiO2-NP–FA and UCP@SiO2-NR–FA

Scheme 1 shows the preparation process of the nanocomposites UCP@SiO2-NP–FA and UCP@SiO2-NR–FA and their application to OECM-1 oral cancer cells. Au NMs were decorated on the UCP@SiO2 after FA modification because of the reaction involving the NHS/EDC coupling reaction, which would affect the stability of Au NMs or cause aggregation. First, NaYF4:Yb3+/Er3+ NPs were synthesized through a high-temperature co-precipitation method with a capping reagent of oleic acid (OA) in octadecene; the average diameter of the NaYF4:Yb3+/Er3+ NPs was approximately 20.6 ± 1.5 nm and their morphology showed a uniform sphere without aggregation. The transmission electron microscopy (TEM) image of NaYF4:Yb3+/Er3+ NPs is shown in Fig. 1a. Moreover, silica shells were coated around the UCPs by a modified reverse water-in-oil microemulsion procedure, and 3-aminopropyl-triethoxysilane (APTES) was used to extend the amino group on the surface of silica to connect to Au NPs or Au NRs. In this process, the zeta potential of UCP@SiO2-NH2 would change from −28 to +23, which proved that the amino group was grafted on the surface. The thickness of the silica shell could be easily controlled at 1.2, 2.2, 4.3, 7.9 and 12.8 nm via reaction with increased amount of tetraethyl orthosilicate (TEOS) (Fig. S1, ESI†). Meanwhile, Fourier transform infrared (FT-IR) spectroscopy was used to confirm the silica shell modification. For UCPs capped with OA, the stretching bands of 2935 and 2859 cm−1 indicated a C–H stretching vibration, and 1566 and 2859 cm−1 were assigned to a COO− stretching vibration.
vibration. After silica shell modification, the strong stretching vibrations of 3465 and 1080 m\(^{-1}\) were expressed as O–H and Si–O–Si, respectively (Fig. S2, ESI†). The crystalline phase of the NaYF\(_4\)-Yb\(^{3+}/\)Er\(^{3+}\) NPs was confirmed as hexagonal according to the reference peak (JCPDS file No. 16-0334). The high-resolution TEM image also showed a lattice fringe of approximately 0.29 nm, which was denoted to be the (101) phase in hexagonal crystalline by powder X-ray diffraction (XRD) (Fig. 1b and c). In UCP systems, NaYF\(_4\) is one of the most commonly used host materials in the upconversion process because of its low non-radiative loss and the ion radius of Y\(^{3+}\) is similar to that of the trivalent ion of lanthanide. Moreover, Yb\(^{3+}\) could sufficiently absorb the energy denoted to 980 nm light because of its 4f level transition of \(^{2}F_{7/2} \rightarrow ^{2}F_{5/2}\). Er\(^{3+}\) was doped to obtain UCLs at 408, 521, 541 and 654 nm, which are attributed to \(^{2}H_{9/2} \rightarrow ^{4}I_{15/2}\), \(^{2}H_{11/2} \rightarrow ^{4}I_{15/2}\), \(^{4}S_{3/2} \rightarrow ^{4}I_{15/2}\) and \(^{4}F_{9/2} \rightarrow ^{4}I_{15/2}\) transitions, respectively. After silica shell coating, the PL intensity decreased by 25% because of the change in the dielectric constant (Fig. 1d).

To determine the optimal conditions for UCP@SiO\(_2\)-Au NM–FA application for PTT and cell imaging, a balanced point for UCL quenching and enhancing should be established, including the effective modification amount of Au NMs and the thickness of the silica shell. Furthermore, the SPR absorption peak of Au NRs should be adjusted at first to match the UCL emission because the 3.5 nm Au NPs already revealed a strong SPR at approximately 525 nm. We tuned the SPR position of Au NRs by adding less silver nitrate during the growth reaction because silver ions play as active sites. Therefore, less silver ions could cause short Au NRs, and thus the SPR was tuned at 525 and 655 nm.\(^{31}\) However, a typical seed-mediated growth method for Au NR synthesis involves a high-cytotoxicity surfactant, such as hexadecyltrimethylammonium bromide (CTAB).\(^{31}\) To reduce the latent hazard of cytotoxicity for Au NRs from CTAB and adjust the surface charge to negative, CTAB should be exchanged with O-carboxymethylchitosan (OCMCS) in Au NRs. Both Au NPs and Au NRs replaced the protecting ligands by OCMCS and showed the same surface property. OCMCS, which has high biocompatibility and could stabilize Au NMs in solution, were prepared in the same method from our previous report.\(^{35}\) Subsequently, different volumes of Au NPs and Au NRs would individually react with various thicknesses of UCP@SiO\(_2\), enabling further study of the influence of the SPR effect. Moreover, the precise Au/Y ratio would be detected by inductively coupled plasma mass spectrometry (ICP-MS). In Fig. 2, the emissions of UCL at 521 and 654 nm for UCP@SiO\(_2\)-NPs and UCP@SiO\(_2\)-NRs were different. For UCP@SiO\(_2\)-NPs, when the silica shell was 1.2 nm thick, the PL intensity decreases by 25% or 50% at 521 nm with 0.86 and 1.42 Au/Y ratios, respectively, and the PL intensity only changes slightly when the silica shell thickness increases, except for the 1.42 Au/Y ratio. Meanwhile, for UCP@SiO\(_2\)-NRs, both emissions at 521 and 654 nm vary with different Au NR loadings and silica shell thickness. At low loading of Au NRs to be 0.21 and 0.84 Au/Y ratios, the UCL intensity could be enhanced by approximately 1.25 or 1.5 times when the silica shell thickness was below 10 nm and decline in all thickness values for 1.41 and 2.59 Au/Y ratios. The conditions for emission at 408 and 541 nm were similar to that at 521 nm, as shown in Fig. S3 (ESI†). Consequently, the SPR effect was the main factor that affects UCL intensity because the noticeably enhanced or quenching phenomena could only be observed in UCP@SiO\(_2\)-NRs. Also, the UCL intensity will be noticeably enhanced or quenching phenomena could only be observed in UCP@SiO\(_2\)-NRs. Therefore, the interaction between the excitation light (980 nm laser) and the coupling effect from Au NRs, because some of Au NRs would be close to each other in random and generate a region with high density of electrons (hot spot), which could potentially cause influence of UCL by interacting with the light source.\(^{24}\) However, this phenomena would only result from the case of Au NRs, because the wavelength of the laser source is not affected by Au NPs.

The minor factor would be the amount of Au NMs; that is, UCL would be increased with a low amount of Au NMs. Moreover, the silica shell was the least factor that influences UCL because the average intensity had no dramatic change. Fig. 3 shows the diversity.
of the SPR effect from Au NPs and Au NRs influencing UCL. The Au NPs can only generate one plasmonic field by exciting the green emission (521 and 541 nm) from the UCPs, but the Au NRs could create two plasmonic fields by exciting both green and red emissions (521, 541 and 654 nm), which would match to induce strong oscillation of the surface electron. In general, the emission intensity would be first quenched because the energy absorbed by Au NMs under certain critical situations could further improve the irradiative process through the SPCE effect.36 For UCP@SiO2-NPs, this phenomenon explained that some UCLs present declining or similar intensities because of the weak interaction from plasmonic enhancement. Moreover, when the Au/Y ratio increased to a high density of cover layer on the UCP@SiO2, the absorption efficiency to UCPs at 980 nm is interrupted and the emission from UCPs is hindered.22

To apply UCP@SiO2-NPs and UCP@SiO2-NRs for PTT, the energy must be sufficiently absorbed by Au NMs because the photothermal effect induces a heating process from a non-radiative oscillation from the surface of electrons. Therefore, the high quantity of Au NMs was a main factor because of the obvious decrease in UCL intensity, which implied an effective energy transfer to Au NMs. In Fig. 4, we considered the Au/Y ratios of 0.84 and 1.41 for UCP@SiO2-NRs (0.86 and 1.42 Au/Y ratios for UCP@SiO2-NPs) that could present a low and high loading amount for the investigation of the photothermal property without fully quenching the UCL intensity. Moreover, 1.2 and 4.3 nm thick silica shells were also used to determine the influence of the distance spacer; these two thicknesses were referred to as near and far distance for investigation. To verify the efficiency of transferring light to heat energy, we performed a simple test, in which the temperature of the solution was monitored during irradiation by a 980 nm laser. In Fig. 5, the silica shells are 1.2 and 4.3 nm thick; in both results, the temperature rapidly increased within 40 s, which slowly decreased with the proportion of growth. For 1.2 nm thickness, only UCP@SiO2-NRs with a 1.41 Au/Y ratio could reach 52 °C. The other samples reached only 36–38 °C, except for water with almost no change, which might be caused by the unfavorable energy transfer during photothermal conversion. Based on Fig. 4, only UCP@SiO2-NRs with a 1.41 Au/Y ratio decreased considerably without enhancement of the UCL intensity. The same result is found for 4.3 nm thick silica shells, in which the obvious difference was caused by the enhanced or quenched UCL emission. The highest temperature was only 43.2 °C for 4.3 nm thick silica shells, and a lower rate of decline was observed for 1.2 nm thick shells. Thus, we suggest that the heating process was the major exhibition under declining UCL emission. To investigate different efficiencies of conversion for light to heat by Au NPs and Au NRs, the integrated areas of UCL emission spectra were compared with the individual systems, which decreased by 33% and 45% after modifying Au NPs and Au NRs, respectively (Fig. S4, ESI†). This result provides an evidence for relatively high energy transfer to heat depending on the SPR absorption.
The emission decay time of the two types of nanocomposites were also measured at 541 and 654 nm, as shown in Fig. 6. At 541 nm, both systems had a decreased PL lifetime after combining with Au NMs; moreover, the PL lifetime decreased from 94 μs to 75.2 and 85.1 μs when the Au/Y ratio was 1.41 and 1.42 for UCP@SiO₂-NPs and UCP@SiO₂-NRs, respectively. At 654 nm, the PL lifetime of the UCP@SiO₂-NRs declined from 249 μs to 167.8 μs with the increase in Au/Y ratio; conversely, the PL lifetime had almost similar values with UCP@SiO₂-NPs, from 249 μs to 245 μs. The decline in UCL intensity and PL lifetime might explain the energy transfer mechanism, as shown in Fig. 3, in which the photon could be absorbed specifically through LRET to Au NMs, and this energy was used to generate heat by a non-radiative process.37

Simulative model for the evaluation of the heating variation

According to the optical characterization and photothermal test, UCP@SiO₂-NRs could possess a higher heating than UCP@SiO₂-NPs because energy was strongly absorbed from three emission bands, which were denoted at 521, 541 and 654 nm. However, the relationship between the plasmonic field and heat quality was still unclear. To determine the basic variation of performance at different wavelength peaks for Au NPs and Au NRs, we used a simulative model built using the finite element method (FEM) for a theoretical study. As shown in Fig. 7 and 8, we fabricated a model consisted of UCP@SiO₂ and one Au NP or Au NR, and the plasmonic field was generated under three emissions at 525, 540 and 655 nm. Moreover, to apply these nanocomposites as bifunctional NMs in PTT and cell imaging, 4.3 nm thick silica shells were selected for the simulative study because of the absence of full UCL emission quenching at a high Au/Y ratio. Fig. 6 shows the distribution of the electric field, which was derived to heat distribution by the time average resistive heating equation. The induced heat was subsequently calculated by integrating the resistive heating on Au NMs. The heat was also produced, depending on the SPR peak of Au NMs (Fig. 7b and 8b). For UCP@SiO₂-NPs, heat could be noticeably generated at green emission only, which indicated the \( ^2H_{11/2} \rightarrow ^4I_{15/2} \) and \( ^4S_{3/2} \rightarrow ^4I_{15/2} \) transitions. The quality of heat was integrated in the whole area displayed on the Au NPs, which were \( 1.55 \times 10^{-14} \), \( 4.83 \times 10^{-14} \) and \( 1.17 \times 10^{-15} \) W at 525, 540 and 655 nm, and the difference between the heat quality at 540 nm and at 525 nm could be observed to be slightly three times more and almost 41 times than that at 655 nm (\( ^4F_{9/2} \rightarrow ^4I_{15/2} \) transition) (Fig. 7c).
Moreover, two factors should be considered for the Au NRs, including the SPR position and orientation of the connecting point, because the Au NRs are anisotropic NMs. For UCP@SiO$_2$-NRs, we discussed the two major directions connecting at head or body of Au NRs (x or y-axis), in which the heat were $1.66 \times 10^{-13}$, $5.92 \times 10^{-13}$ and $4.54 \times 10^{-13}$ W for the x-axis, and $2.53 \times 10^{-13}$, $8.92 \times 10^{-13}$ and $5.24 \times 10^{-13}$ W for the y-axis at 525, 540 and 655 nm (as shown in Fig. 8c and d). Also, the heat distribution at 655 nm was higher by 5.74 and 1.67 times than that at 525 nm and 540 nm, respectively, for the modification at the x-axis; conversely, heat at 540 nm was higher by 3.53 and 1.6 times than that at 525 nm and 655 nm for the y-axis, respectively. We accumulated all the integrated heat, including 525, 540 and 655 nm, and found almost the same values as $1.69 \times 10^{-12}$ W and $1.67 \times 10^{-12}$ W for the x-axis and y-axis of the connecting point. This phenomenon can be explained by Fig. 8b, in which the total heat value calculated from the two bands (green and red) was similar at around 40 W. NaYF$_3$:Yb$^{3+}$/Er$^{3+}$ NPs could also emit stronger green emission than red; when Au NRs were conjugated with UCPs at the y-axis, the efficient oscillations of the electric field from green light absorption ($^{2}S_{3/2} \rightarrow ^{4}I_{15/2}$ and $^{4}S_{3/2} \rightarrow ^{4}I_{15/2}$) were the major affected radiation in heat production. However, when Au NRs conjugated with UCPs by head point, red emission ($^{2}F_{9/2} \rightarrow ^{4}I_{15/2}$) could be the major induced heat of radiation. This variation resulted in the strong heating process at the weak red emission region, which corresponded to the high SPR absorption peak (655 nm). Meanwhile, comparison of the total integrated heat between the Au NP and Au NR systems showed that the UCP@SiO$_2$-NRs could efficiently generate more heat than the UCP@SiO$_2$-NPs by almost 26 times because better interaction between SPR and UCL existed in the former.

**UCP@SiO$_2$-NP–FA and UCP@SiO$_2$-NR–FA for PTT in vitro**

According to the chemical, physical and theoretical characterization, the most suitable nanocomposite for oral cancer PTT between conjugation to Au NPs or Au NRs is the UCP@SiO$_2$-NRs because of the predominant performance of photothermal energy transfer. Moreover, the FA molecules were covalently bonded with –NH$_2$ on the silica surface to improve the targeting ability for cancer cells and the stability in the aqueous solution. In this study, we selected OECD-1 oral cancer cells to demonstrate PTT efficiency. OECD-1 is a kind of oral epidermoid carcinoma cell line. PTT treatment is better than chemotherapy because the latter has significant side effects and challenges. To eliminate the cytotoxicity of these nanocomposites to OECD-1 without laser treatment, we first incubated different concentrations of nanocomposites with OECD-1 for 24 and 72 h. In Fig. 9a and b, no obvious cytotoxicity was observed for UCP@SiO$_2$–FA; also after modification with Au NMs, the cell viability still remained above 90%, and the nanocomposite concentration was increased from 1 to 250 $\mu$g mL$^{-1}$ in both 24 and 72 h treatment. Moreover, FA has been widely used for improving the intracellular uptake through caveolin-mediated endocytosis, and confocal microscopy was used to verify the effect of FA modification in improving the binding affinity to cancer cells.$^{38,39}$ As shown in Fig. 9c, UCP@SiO$_2$-NR–FA are highly accumulated into OCEM-1 and most of the nanocomposites are located around nuclei because they are too large to enter into the nuclei. Moreover, the improvement in accumulation could be attributed not only by FA modification but also by the adverse interaction between nanocomposites and OECD-1 because the zeta potential of these nanocomposites and the cell membrane were both negative. We then examined the therapeutic efficiency of both UCP@SiO$_2$-NP–FA and UCP@SiO$_2$-NR–FA with low and high loading amounts of Au NMs via irradiation at 980 nm laser with 1.5 W cm$^{-2}$ (conservative limit set for human skin exposure to 980 nm NIR light is 0.7 W cm$^{-2}$, ANSI Z136 Standards, http://www.lia.org/publications/ansi) for 1 min. Trypan blue was immediately used to stain the treated cell for quick PT efficiency test. In Fig. 10, OECD-1 oral cancer cells were killed and a considerable difference was found between UCP@SiO$_2$-NP–FA and UCP@SiO$_2$-NR–FA, which is attributed to the discrepancy of heating ability and heat distribution. According to the simulative results from Fig. 7 and 8, most of the heat was generated at the contact surface between the UCPs and the Au NMs, but the Au NRs could reveal preferable heating area because of their unique
anisotropic properties and large surface area. The Au NPs, Au NRs and UCP@SiO$_2$ were also treated with OEMC-1 for the control group, and the results showed no observable cell death after irradiation with a 980 nm laser (Fig. S6, ESI†). Compared with the UCP@SiO$_2$-NP–FA with weak therapeutic performance, most of the cells were dead in UCP@SiO$_2$-NR–FA treatment with a 1.41 Au/Y ratio, which showed excellent potential for PIT in OE CM-1 oral cancer cells.

4. Conclusions

In this study, we determined the optimal conditions for the combination of UCPs and Au NMs as bifunctional nanocomposites in the treatment of OE CM-1 oral cancer cells via PTT and cell imaging. First, UCP@SiO$_2$-NP–FA and UCP@SiO$_2$-NR–FA were prepared via a multi-step process, and the size, morphology, and crystalline phase of the fabricated nanocomposites were confirmed by TEM, high-resolution TEM and powder XRD. The mechanism and efficiency of energy transfer from UCPs to Au NMs were investigated via characterization of the variation of UCL PL intensity and decay time emission. A simulative model for the Au NP and Au NR systems was used to study the heat quality and heat distribution generated by green and red UCL emissions under laser irradiation at 980 nm. Moreover, in vitro studies showed the non-cytotoxicity of the nanocomposites before exposure to 980 nm laser and revealed an exceptional therapeutic efficiency via photothermal effects. Based on the results, the highly potent nanocomposites for PTT and cell imaging are the UCP@SiO$_2$-NR–FA with a 4.3 nm thick silica shell and a 1.41 Au/Y ratio.

Acknowledgements

The authors would like to thank the Ministry of Science and Technology of Taiwan (Contract No. MOST 104-2113-M-002-012-MY3, 103-2745-M-002-004-ASP and 103-2911-I-002-594) and Academia Sinica (Contract No. AS-103-TP-A06) for financially supporting this research. This work is also supported by the NSFC (No. U1305244 and 21325104) and the CAS/SAFEA International Partnership Program for Creative Research Teams. Thanks to Ms C. Y. Chien of the Precious Instrument Center (National Taiwan University) for the assistance in TEM experiments and Ms Li-Wen Lo of The Genomics Research Center, Academia Sinica for the help with the confocal microscopy. D. P. Tsai also grateful to National Center for Theoretical Sciences, Molecular Imaging Center of National Taiwan University, National Center for High-Performance Computing, Taiwan, and Research Center for Applied Sciences, Academia Sinica, Taiwan for their support.

Notes and references


