Gold nanorods-conjugated TiO$_2$ nanoclusters for the synergistic combination of phototherapeutic treatments of cancer cells

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Abstract

Background: Recently, a combination of photodynamic therapy (PDT) and photothermal therapy (PTT) to generate reactive oxygen species (ROS) and heat to kill cancer cells, respectively has attracted considerable attention because it gives synergistic effects on the cancer treatment by utilizing the radiation of nontoxic low-energy photons such as long wavelength visible light and near IR (NIR) penetrating into subcutaneous region. For the effective combination of the phototherapies, various organic photosensitizer-conjugated gold nanocomplexes have been developed, but they have still some disadvantages due to photobleaching and unnecessary energy transfer of the organic photosensitizers.

Results: In this study, we fabricated novel inorganic phototherapeutic nanocomplexes (Au NR–TiO$_2$ NCs) by conjugating gold nanorods (Au NRs) with defective TiO$_2$ nanoparticle clusters (d-TiO$_2$ NP clusters) and characterized their optical and photothermal properties. They were observed to absorb a broad range of visible light and near IR (NIR) from 500 to 1000 nm, exhibiting the generation of ROS as well as the photothermal effect for the simultaneous application of PDT and PTT. The resultant combination of PDT and PTT treatments of HeLa cells incubated with the nanocomplexes caused a synergistic increase in the cell death compared to the single treatment.

Conclusion: The higher efficacy of cell death by the combination of PDT and PTT treatments with the nanocomplexes is likely attributed to the increases of ROS generation from the TiO$_2$ NCs with the aid of local surface plasma resonance (LSPR)-induced hot electrons and heat generation from Au NRs, suggesting that Au NR–TiO$_2$ NCs are promising nanomaterials for the in vivo combinatorial phototherapy of cancer.

Keywords: Phototherapeutic nanocomplexes, TiO$_2$ nanoclusters, Gold nanorods, HeLa cells, Cancer therapy, Photodynamic therapy, Photothermal therapy
the immediate locale of the light-absorbing PS or PTA which can be activated only in the particular areas of cancer cells/tissues that have been exposed to light. The most of the PSs used in cancer therapy are organic dyes such as porphyrin derivatives [7, 12–15], boron-dipyromethene (BODIPY) conjugates [16] and methylene blue [17]. However, the PDT using the organic PSs gives unsatisfactory results because the PSs absorb visible light mostly [18] with limited ability of near IR (NIR) (650–900 nm) penetrating deeply into biological tissue, and the PDT alone is not suitable for subcutaneous treatment. Thus, for the subcutaneous treatment, PTT using gold nanoparticles has been attracting interests because gold nanoparticles can absorb NIR radiation to generate heat killing cancer cells [11, 19–22]. Nevertheless, the efficacy of PTT is not so high as compared to that of PDT. Therefore, many researchers have attempted to apply the combination of PDT and PTT to enhance the therapeutic efficiency synergistically against malignant carcinomas as compared to PDT or PTT alone [10, 23–25].

For the effective combination of the phototherapies, many PS-conjugated PTA nanocomplexes have been developed. However, ROS generation by PS is more or less inhibited due to the excitation energy transfer from PS to PTA in addition to oxygen, and the combination efficiency cannot be maximized as anticipated. In order to overcome this problem, modification of the PS–PTA nanocomplexes has been performed by leaving the space between PS and PTA [7, 26–28]. Very recently Chung et al. [7] have prepared the dendrimer porphyrin (DP)-coated gold nanoshell (DP-AuNS) in which dendritic wedges of DP play a role as a spacer between porphyrin and PTA to minimize the additional energy transfer, and they found that the DP-AuNS could be applied to synergistic combination of the PDT and PTT. In spite of such improvements of PS–PTA nanocomplexes, they can’t be used for a long time because of photobleaching of organic photosensitizers. Thus, inorganic semiconductor nanomaterials would be rather useful as an alternative PS if they generate ROS. Among the semiconductor nanomaterials, TiO2 NPs are attracting much attention as an alternative PS if they generate ROS. Among the semiconductor nanomaterials, TiO2 NPs are attracting much attention as an alternative PS if they generate ROS. Among the semiconductor nanomaterials, TiO2 NPs are attracting much attention as an alternative PS if they generate ROS. Among the semiconductor nanomaterials, TiO2 NPs are attracting much attention as an alternative PS if they generate ROS.

Results

Preparation and characterization of Au NR–TiO2 NCs

The fabrication processes of new photo-functional nanocomplexes for the combination of phototherapeutic treatments are illustrated in Fig. 1. Firstly, d-TiO2 NPs were synthesized by hydrothermal reaction of liposome-TiO2 composites as previously reported [18], and their surfaces were modified by binding with APTES. In parallel with this procedure, the citrates capped on the surfaces of Au NRs were exchanged with HS–PEG–COOH. Then, preparation of the Au NR–TiO2 nanocomplexes (Au NR–TiO2 NCs) was performed by coupling of the surface-modified nanomaterials to lead the formation of the amide bond between the amine group of APTES–TiO2 NPs and the carboxyl group of PEG–Au NRs [37, 38].

Figure 2A (a) and (b) show the TEM images of the as-prepared APTES–TiO2 NPs (average diameter of ~25 nm) and PEG–Au NRs (average aspect ratio of ~4, 100 nm length and 25 nm diameter), respectively. In fact, APTES–TiO2 NPs in aqueous solution was observed to agglomerate together to form clusters. The average diameter of a cluster was evaluated to be about 224.2 nm by dynamic light scattering (DLS) measurement (Additional file 1: Figure S1). The cluster size was observed to be increased by about 90 nm after mixing with PEG–Au NRs as shown in Fig. 2A (c), indicating the formation of the Au NR–TiO2 NCs by coordinating between APTES and PEG. The formation of the Au NR–TiO2 NCs was also confirmed with the diffuse reflectance UV–visible absorbance measurement at room temperature. APTES–TiO2 NPs exhibited a broad visible light absorption band (500–800 nm) in addition to UV absorption band [Fig. 2B (a)] due to the surface defects as previously reported [7], and PEG–Au NRs exhibited two localized surface plasma resonance (LSPR) absorption peaks at 510 nm
Fig. 1 Schematic illustration of the preparation of Au NR–TiO₂ NCs
and 800 nm. The LSPR absorption peaks shifted to longer wavelengths at 525 nm and 850 nm upon mixing PEG–Au NRs with APTES–TiO₂ NPs. This supports that the Au NRs are conjugated with d-TiO₂ NPs, and the final product of Au NR–TiO₂ NCs could absorb broad visible light as well as NIR light. When Au NR–TiO₂ NCs were dispersed in deionized water at pH 7.0 at a concentration of 1 mg mL⁻¹, they were found to have an average diameter of 317 nm which is appropriate size for their efficient uptake into cells as is within the known range from about 50 to several hundred nanometers (Additional file 1: Figure S1) [39].

In addition to the size of nanocomplexes their surface charges are also known to be an important factor influencing the cell uptake efficiency. Generally, positively charged particles are taken up better than negatively charged ones because the cell plasma membrane is negatively charged [40–42]. In order to estimate the surface charges, the ζ-potentials of different nanoparticles including Au NR–TiO₂ NCs were measured (Fig. 3 and Additional file 1: Figure S2). As shown in Additional file 1: Figure S2, the average ζ-potential of d-TiO₂ NPs in deionized water at pH 7.0 was −21.7 mV, indicating that the surfaces of d-TiO₂ NPs are negatively charged. However, the ζ-potential of APTES–TiO₂ NPs was significantly changed to +16.0 mV. This may be due to ionization of –NH₂ groups of APTES to –NH₃. On the other hand, the ζ-potential of Au NRs was −12.5 mV even after surface
modification with PEG, but this negative potential was observed to be changed to a positive value of +6.8 mV upon mixing with APTES–TiO2 NPs, supporting again that d-TiO2 NPs are bonded to the Au NRs. Therefore, the as-synthesized Au NR–TiO2 NCs are anticipated to be easily taken up to the cellular membranes and entered into the cytoplasm probably via endocytosis [43].

**Intracellular generation of ROS from nanoparticles**

To evaluate the generation of intracellular generation of ROS from the nanoparticles in cancer cells, firstly HeLa cells were incubated with 100 μg/mL of three different nanoparticles (APTES–TiO2 NPs, PEG–Au NRs, and Au NR–TiO2 NCs) and their confocal images were measured. Figure 4a shows the confocal DIC images which were compared with blue fluorescent images of DAPI (4,6-diamidino-2-phenylindole), a nucleus stain of HeLa. Comparison of these images revealed that cytoplasm of the cells treated with APTES–TiO2 NPs or Au NR–TiO2 NCs exhibited dark spots in contrast to clear cytoplasm of the untreated or PEG–Au NRs, indicating that PEG–Au NRs are not internalized into the cells whereas APTES–TiO2 NPs or Au NR–TiO2 NCs are easily entered into the cells by endocytosis [44]. This is consistent with the fact that APTES–TiO2 NPs or Au NR–TiO2 NCs have positive ζ-potential for the efficient cell uptake while PEG–Au NRs have negative ζ-potential as aforementioned. It is also noteworthy that similar cell morphology was retained in the dark whether or not treated with nanoparticles, indicating no major effect of nanoparticles themselves on cell viability (Additional file 1: Figure S3 (A)).

The intracellular ROS generation was examined by monitoring green fluorescence from a standard ROS probe [40], H2DCFDA upon visible light irradiation. A strong green fluorescence was observed in the cytoplasm of HeLa cells treated with APTES–TiO2 NPs and Au NR–TiO2 NCs while not observed in untreated cells, indicating that the nanoparticles were taken up and internalized to generate ROS upon irradiation. Quantification of ROS based on fluorescence intensities (Fig. 4b) revealed that the significantly high level of ROS was produced by APTES–TiO2 NPs and Au NR–TiO2 NCs as compared with negligible level generated by PEG–Au NRs. It is noteworthy that such green fluorescence was not observed from any nanoparticles-treated cells upon irradiation with NIR at 808 nm [Additional file 1: Figure S3 (B)]. These results demonstrate that the observed ROS generation in the cells was resulted from the absorption of the visible light by TiO2 NPs. Therefore, the relatively lower level of ROS produced from Au NR–TiO2 NCs is attributed to inhibition of directly visible light absorption of NPs by the LSPR of Au NRs or the LSPR-enhanced charge transfer quenching of the visible-light-induced electrons which are supposed to produce ROS [45]. Anyway, it is evident that Au NR–TiO2 NCs are able to not only generate the significant amount of ROS but also induce photothermal heating from Au NRs exposed to NIR light which is caused by electron energy loss due to its longitudinal LSPR oscillation [46]. Thus, it would be worthwhile to attempt to combine both photodynamic and photothermal effects with Au NR–TiO2 NCs using visible light and NIR.

**Photothermal properties of nanoparticles and nanocomplexes**

In order to examine the photothermal heating in the nanoparticles (nanocomplexes)-containing HeLa cells, the temperature of the cell culture solution was measured with infrared camera upon irradiation using an 808 nm NIR laser (1 W/cm²) and a xenon lamp. Figure 5A shows the temperature changes of the solution containing 100 μg mL⁻¹ of three different nanoparticles such as APTES–TiO2 NPs, PEG–Au NRs and Au NR–TiO2 NCs as a function of NIR irradiation time. The highest temperature was reached up to 72 °C from 23 °C rapidly 5 min after irradiation on PEG–Au NRs and the medium temperature change was observed up to 45 °C with Au NR–TiO2 NCs in contrast to negligible temperature change with APTES–TiO2 NPs. However, such temperature changes were not significantly observed upon irradiation with visible light (Fig. 5b). These results indicate that the photothermal heating is induced by Au NRs in Au NR–TiO2 NCs upon exposure to NIR. Such photothermal heating could be observed even by using the small amount of Au NR–TiO2 NCs (Additional file 1: Figure S4).
In vitro cell viability under the dark and light irradiation conditions

The viability of HeLa cells with different treatments was estimated by evaluating their cytotoxicity effect on Hela cells using the EZ-Cytox reagent based on the water-soluble tetrazolium (WST) method. For the control experiments, all the cells were incubated with nanoparticles such as APTES–TiO$_2$ NPs, PEG–Au NRs, and Au NR–TiO$_2$ NCs under the dark condition for 2 days, and they were observed to survive almost completely like

![Fig. 4](image-url)
the untreated cells. Figure 6a (left) demonstrates that all the nanoparticles showed good cytocompatibility (>98%) over various concentrations from 10 μg mL$^{-1}$ up to 100 μg mL$^{-1}$ in dark (Fig. 6b). In contrast to the high cell viability under the dark condition, the HeLa cells incubated with 100 μg mL$^{-1}$ of APTES–TiO$_2$ NPs, PEG–Au NRs or Au NR–TiO$_2$ NCs exhibited extensive cell death upon photoirradiation as shown in Fig. 6a (right), even though the viabilities of the cells without nanoparticles were not affected by the sequential irradiation of visible light for 30 min and NIR for 5 min. As shown in Fig. 6c, the photoinduced cell death was observed to depend on the concentrations of nanoparticles or nanocomplexes under different photoirradiation conditions, starting even with the low dose (10 μg mL$^{-1}$) and further increase with the concentration increased to 100 μg mL$^{-1}$, indicating that all the three nanoparticles photocatalyzed the killing of cancer cells under the photoirradiation.

**Discussion**

The observed cell death with APTES–TiO$_2$ NPs or PEG–Au NRs was caused by single irradiation of visible light or NIR respectively, suggesting that APTES–TiO$_2$ NPs or PEG–Au NRs make the most of PDT or PTT effects separately as the maximum ROS or heat was generated from APTES–TiO$_2$ NPs or PEG–Au NRs, respectively under the single irradiation of visible light or NIR (Additional file 1: Figure S4 and Fig. 5). Nevertheless, their efficacy of the photoinduced cell death was observed to be similar and low with about 20–30% killing at a concentration of 100 μg mL$^{-1}$. On the other hand, about two times higher cell death (30% cell viability) was achieved from the cells incubated with the same concentration of the nanocomplex, Au NR–TiO$_2$ NCs upon simultaneous irradiation of visible light and NIR using the irradiation set up shown in Additional file 1: Figure S5, even though ROS or heat generated from Au NR–TiO$_2$ NCs upon single irradiation of visible light or NIR is rather two times lower than those from APTES–TiO$_2$ NPs or PEG–Au NRs (Figs. 4 and 5). Particularly it should be noted that from the confocal microscopic images of the cells incubated with Au NR–TiO$_2$ NCs (Fig. 7) much stronger green fluorescence was observed to emit as a result of the simultaneous irradiation rather than the visible light irradiation alone. These results imply that the higher efficacy of cell death in the presence of Au NR–TiO$_2$ NCs is likely due to the visible-light-induced ROS generation enhanced synergistically by the heat generated by NIR irradiation, considering that the temperature-dependent (up to 40 °C) increases of ROS generation had been observed in the process of the aminolaevulinic acid (ALA)-treated PDT of human skin fibroblasts [47].

Thus, the mechanism of the photokilling of cancer cells with Au NR–TiO$_2$ NCs can be proposed as illustrated in Fig. 8. When both visible light (420–780 nm) and NIR are irradiated on Au NR–TiO$_2$ NCs, the visible and NIR photons are absorbed by d-TiO$_2$ NP and Au NR, respectively. The visible light energy separate electrons and holes in the defective states and the valence band (VB) of d-TiO$_2$ NPs, respectively. The electrons in the defective states can react with oxygen molecules in the solution to produce a certain amount of superoxide radicals.
as one of ROS to be used for the cell killing while the photogenerated holes in VB can react with H₂O to form hydroxyl radicals (·OH) as another type of ROS. On the other hand, the NIR irradiation causes LSPR excitation of Au NRs are known to form hot electrons which are injected into the conduction band (CB) of the conjugated TiO₂ NPs with photogenerated holes left in Au NRs [48, 49]. Such an interfacial electron transfer from Au to TiO₂ in the nanocomplex would be further facilitated by overcoming Schottky barrier through the NIR-induced heat generation from the Au NRs as reported in other Au–TiO₂ nanohybrids which are applied to visible light photocatalysts for the destruction of pollutants [50–53]. Consequently, more O₂⁻ can be produced by the reaction of oxygen molecules with the injected electrons in the CB of TiO₂ NPs through the LSPR excitation. Also, the holes in Au NRs also produce additional ·OH. Therefore, we can conclude that the simultaneous irradiation with visible light and NIR on Au NR–TiO₂ NCs produce ROS synergistically from d-TiO₂ NPs with the aid of LSPR-induced electron and heat generation from the conjugated Au–NRs, and we believe that the nanocomplex Au NR–TiO₂ NCs are a greatly potent phototherapeutic nanomaterials for the improved application of combination of PDT and PTT to the cancer therapy.

**Conclusion**

Significant PDT and PTT effects were observed from HeLa cells treated with d-TiO₂ NPs or Au NRs upon visible light or NIR irradiation alone. Further synergistic enhancement
of the phototherapeutic efficiency was achieved by simultaneous irradiation with visible light and NIR onto the cells incubated with inorganic nanocomplex such as Au NR–TiO₂ NCs which were newly fabricated by coupling d-TiO₂ NPs and Au NRs. It was found that visible light-induced ROS production from the Au NR–TiO₂ NCs increased with the aid of LSPR-induced hot electrons and heat generation. Therefore the combination of PDT and PTT treatments with Au NR–TiO₂ NCs has a great potential to be applied to improve the cancer therapy.

**Materials and experimental methods**

**Chemicals**

All of the chemicals used for the synthesis of AuNR–TiO₂ NPs, including P-25 (Degussa-Huls), egg lecithin (L-R-phosphatidylcholine, > 60%, Sigma-Aldrich), chloroform (> 99.8%, Samchun, Korea), sodium hydroxide (> 93%, Duksan, Korea), ethanol (200–proof, > 99.8%, Sigma-Aldrich), (3-aminopropyl)triethoxysilane (> 99%, Sigma-Aldrich), citrates-capped Au NRs (AC12-25-808-CIT-DIH-1, Nanopartiz), HS–PEG–COOH (MW 10 k, Nanocs), EDC (N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride, > 98%, Sigma-Aldrich) and NHS (N-hydroxysuccinimide, > 98%, Sigma-Aldrich) were of analytical grade and used as purchased without further purification.

**Synthesis of Au NR–TiO₂ NCs**

Firstly, the surfaces of as-prepared d-TiO₂ NPs were modified by binding with APTES as per the previous report [18]. In the first step, APTES solution was prepared by dissolving 0.1% acetic acid, 4% deionized water and 2% APTES in ethanol. The d-TiO₂ NPs were dispersed in a beaker containing 10 mL ethanol under constant ultrasonication for 30 min. Then, the APTES solution was added into the ethanol dispersed d-TiO₂ NPs solution and stirred for 24 h. The solution was washed with ethanol and deionized water several times by repeated centrifugation. An aliquot of these particles was dried in an oven at 40 °C. Next, the purchased citrates-capped Au NRs were exchanged with HS–PEG–COOH to prepare the PEG–Au NRs. Finally, the preparation of the Au NR–TiO₂ NCs was performed by coupling of the two surface-modified nanomaterials (APTES–TiO₂ NPs and PEG–Au NRs) to lead to the formation of the amide bond between the amine group of APTES and the carboxyl group of PEG [37, 38].

**Structural and optical characterization of nanoparticles**

The morphology of the as-prepared d-TiO₂ NPs, PEG–Au NRs and Au NR–TiO₂ NCs were examined by transmission electron microscopy (TEM; Tecnai G2 F30). The TEM sample was prepared by dip-coating Formvar/carbon film–Cu grids with a nanocolloidal solution obtained by sonication of the synthesized nanoparticles in ethanol.

The particle sizes and ζ-potentials of the dispersed solution of the synthesized nanoparticles were measured respectively by dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) using an electrophoretic light scattering spectrophotometer (Otsuka Electronics Co., Ltd; ELS-Z2).

For the optical properties, diffuse reflectance UV–VIS–NIR absorption spectra (DRS) were recorded using a Solid Spec-3700 double beam spectrophotometer equipped with an integrating sphere.
Cell culture and imaging

The human cervical carcinoma cells (HeLa) were grown in 89% Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and 1% antibiotic–antimycotic solution. The cells were routinely maintained in the plastic tissue culture dishes at 37 °C under a humidified 5% CO₂-containing an atmosphere.

The cell images were obtained by observing differential interference contrast (DIC) and fluorescence of DAPI (4′,6-diamidino-2-phenylindole) or H₂DCFDA using laser-scanning confocal microscopy (LSCM; LSM5 live configuration Vario Two VRGB).

Measurement of ROS generation under visible region

For the measurement of ROS generation, HeLa cells were seeded in a 15 μ-slide 8 well plate (Ibidi, Germany) at a density of 5 × 10⁴ cells per well and incubated for 24 h at 37 °C under a 5% CO₂ atmosphere followed by addition of 10 μg/mL of APTES–TiO₂ NPs, PEG–Au NRs or Au NR–TiO₂ NCs. After 24 h, the culture medium was replaced by the new medium contained 20 μg/mL carboxy-H₂DCFDA (Sigma-Aldrich) for 30 min in the conventional incubator (37 °C, 5% CO₂). Next, carboxy-H₂DCFDA-containing medium was removed and added the fresh medium. Then, the 8 well plates were irradiated under broadband visible-light region (12 mW/cm²) with a xenon lamp (Asahi Spectra, MAX-302, Japan) for 30 min. Subsequently, the nucleus was stained with 10 μg/mL bisbenzimide trihydrochloride (Hoechst33342) for 10 min and washed with DPBS several times. Fluorescence images of the intracellular ROS generation detected by carboxy-H₂DCFDA D were obtained with a confocal laser microscope (Zeiss LSM5 live configuration Vario two VRGB).

Photothermal properties of nanoparticles

To measure the photothermal conversion performance of APTES–TiO₂ NPs, PEG–Au NRs and Au NR–TiO₂ NCs, temperature of the nanoparticles-containing solutions were measured with infrared camera (640 × 512 cooled InSb IRFPA with 90 μm pixel pitch) after irradiation using a 808 nm NIR laser (1 W/cm²) or broadband visible light from a xenon lamp (12 mW/cm²).

Evaluation of cytotoxicity and cell viability

The cytotoxicity of the injected nanoparticles was evaluated using the EZ-Cytox reagent (Daeil Lab Service, Seoul, South Korea) based on the water-soluble
tetrazolium (WST) method. HeLa cells were seeded at a density of $1 \times 10^4$ cells per well in a 96-well micro-assay plate and incubated for 24 h at 37 °C under a 5% CO$_2$ atmosphere. The APTES–TiO$_2$ NPs, PEG–Au NRs or Au NR–TiO$_2$ NCs were added to the incubated cells at various concentrations, followed by further incubation for an additional 24 h at 37 °C. Next, 10 µL of the EZ-Cytotox reagent was added and the plates were incubated for 2 h at 37 °C. The absorbance of the EZ-Cytotox reagent was measured at 450 nm using a microplate reader (VersaMax, Molecular Devices, USA). The cell viability (%) was calculated using the following equation: cell viability (%) = $(OD_{450\text{sample}}/OD_{450\text{control}}) \times 100$. The control condition was maintained with cells not treated with anything, neither nanoparticles nor visible/NIR light.

**Photoirradiation method**

The HeLa cells incubated with APTES–TiO$_2$ NPs, PEG–Au NRs or Au NR–TiO$_2$ NCs were irradiated for 30 min with the visible light (12 mW/cm$^2$) emitted from a xenon lamp (Asahi Spectra, MAX-302, Japan) or/and 5 min with an 808 nm NIR laser (1 W/cm$^2$) (see Additional file 1: Figure S5).

**Additional file**

Additional file 1: Figure S1. Sizes of APTES-TiO$_2$ NPs (A), PEG-Au NRs (B) and Au NR-TiO$_2$ NCs (C) were measured in deionized water at pH 7.0 by dynamic light scattering (DLS). Figure S2. Surface charge or zeta potential of TiO$_2$ NPs (A), Au NRs (B) and Au NR-TiO$_2$ NCs (C) were measured in deionized water at pH 7.0 by laser doppler velocimetry (LDV). Figure S3. Laser scanning confocal microscopy images of HeLa cells. DIC images and fluorescence images indicating ROS generation with nanoparticles in the dark (A) or under NIR (808 nm laser) light irradiation (B). Figure S4. Temperature changes of the cell-culture solutions containing various concentrations of APTES-TiO$_2$ NPs (A), PEG-Au NRs (B) and Au NR-TiO$_2$ NCs (C) with various as a function of NIR (808 nm laser) exposure time. Figure S5. Schematic illustration of the optical system for light irradiation.

**Authors’ contributions**

KSC led the project at KBSI, reviewed literature with JL and MY, designed the experimental processes. JL, YHL, and CBJ performed the experiments, collected the data which were analyzed by JL, JSC, KSC and MY. JL, KSC and MY prepared the manuscript and all the authors contributed to the critical reading of the manuscript. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article and its Additional files.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

No animal experiment, and not applicable.

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