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Thiolated Dextran-Coated Gold Nanorods for Photothermal Ablation of Inflammatory Macrophages

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Thiolated dextran-coated gold nanorods (DEX-GNRs) were synthesized for targeted delivery to inflammatory macrophages and their photothermal ablation under near-infrared (NIR) light irradiation. Successful synthesis of DEX-GNRs was achieved using thiolated dextran, generated by applying mercaptopropionic acid to transform a hydroxyl group of dextran into a thiol group which has strong binding affinity with surfaces of GNRs. We confirmed both the existence of a thiol group in the functionalized dextran using Ellman's reagent in a thiol group assay and the characteristic band of DEX-GNRs using FT-IR spectrum. Furthermore, a cellular uptake study revealed that dextran showed a superior ability to bind the GNRs surface against macrophages compared to those of PEGylated GNRs with various molecular weights of polyethyleneglycol (PEG). Consequently, an in vitro phothothermal irradiation experiment using NIR light indicated that DEX-GNRs exhibited a significant cell-killing efficacy, even with a lower concentration of Au and a low-power light source.

Introduction

Inflammatory macrophages play a key role in the development of atherosclerosis because cyto-/chemokines and proteases are released from macrophages and can exacerbate and accelerate damage to normal vessels.[1] Thus, the detection of inflammatory macrophages in the diseased site and their ablation has emerged as a primary target for atherosclerosis therapy.

Recently, localized photothermal therapy has been studied for target cell ablation because the sudden generation of heat can promote denaturation and disruption of intracellular organized biomolecules.[2−5] The key point of photothermal therapy is to destroy the target tissues without damaging the surrounding normal tissues. One promising photothermal approach is the combination of gold nanorods (GNRs) as photothermal agents and a near-infrared (NIR) laser because the longitudinal surface plasmon band of GNRs is located in the NIR region. Near-infrared lasers can penetrate normal tissue with sufficient intensity and spatial precision, allowing them to be used for localized photothermal therapy. It has been shown that photon excitation of surface plasmon bands by NIR light generates excited states in the surface free electrons of GNRs; phonons are then released after relaxation of the electrons. The phonons subsequently relax and are converted to heat within 100 ps.[6−8] Consequently, most photons absorbed by GNRs are transformed into heat for photothermal therapy.

For targeted delivery to macrophages, it is essential to introduce a targeting moiety such as an antibody or polysaccharide onto the nanoplatform.[9,10] Dextran is considered to be an adequate molecule for specific targeting of macrophages with highly biocompatible characteristics. The previous studies on dextran-coated nanoparticles have demonstrated preferential uptake in the cytoplasm of macrophages through dextran receptors or scavenger receptors.[11,12] Moreover, dextran is a superior stabilizer of GNRs in comparison to hexadecyltrimethylammonium bromide (CTAB), a kernel ligand in the GNR formulation process.[13]

Thus, we synthesized thiolated dextran-coated gold nanorods (DEX-GNRs) for targeted delivery to inflammatory macrophages and utilized the photothermal ablation of inflammatory macrophages under NIR laser irradiation. A conceptual scheme of this study is provided in Figure 1. To prepare DEX-GNRs, monodispersed GNRs were synthesized, and their surfaces were modified with thiolated dextran. We compared the efficiencies in cellular uptake and in macrophage photothermal ablation of DEX-GNRs and PEGylated GNRs (PEG-GNRs) with various molecular weights of polyethyleneglycol (PEG). The cytotoxicity of the DEX-GNRs was also evaluated using a cell proliferation assay.

Experimental Methods

Materials. Gold(III) chloride trihydrate (HAuCl4·3H2O), hexadecyltrimethylammonium bromide (CTAB), sodium borohydride,

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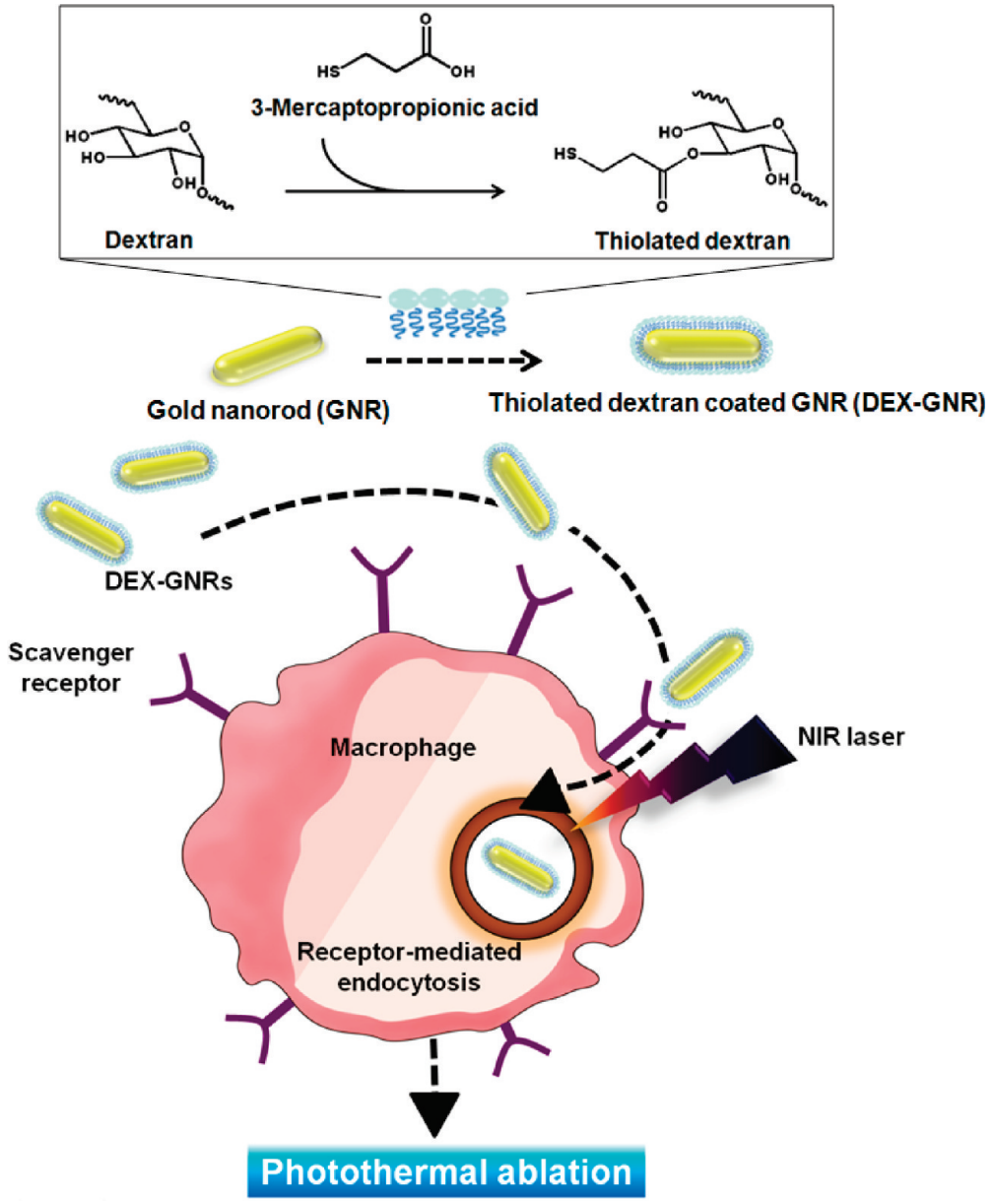

Figure 1. Schematic illustration of thiolated dextran-coated gold nanorods (DEX-GNRs) synthesis.

silver nitrate, L-ascorbic acid, dextran (M_w 10K Da), 1,3-dicyclohexylcarbodiimide, 4-(dimethylamino) pyridine, 3-mercaptopropionic acid, and dimethyl sulfoxide were obtained from Sigma-Aldrich (St. Louis, USA). Thiol-poly (ethylene glycol) monomethyl ether (thiolated PEG, M_w 2K, 5K, and 10K Da) was purchased from Sunbright (Japan). Dulbecco's phosphate buffered saline (PBS, pH 7.4) was obtained from Hyclone (Logan, UT), and 4-(3-(4-lodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio)-1,3-benzene disulfonate (WST-1) was purchased from Takara (Japan). Ultrapure deionized water was used for all of the syntheses. All other chemicals and reagents were analytical grade.

Synthesis of Thiolated Dextran-Coated Gold Nanorods (DEX-GNRs). Thiolated dextran (M_w 10K Da) was synthesized by dissolution of dextran (0.3 mol, 3 g), 3-mercaptopropionic acid (6.0 mol, 636.8 mg), 1,3-dicyclohexylcarbodiimide (9.0 mol, 910.7 mg), and 4-(dimethylamino)pyridine (9.0 mol, 1099.5 mg) in dimethyl sulfoxide (50 mL). The reaction was carried out for 48 h at room temperature. After lyophilization of the solvent, a mixture of reactants and products was dissolved in 10 mL of deionized water. Water-insoluble 1,3-dicyclohexylcarbodiimide was removed by filtration, and surplus 4-(dimethylamino) pyridine was dialyzed for a week. Thiolated dextran was finally obtained after lyophilization of deionized water.

Gold nanorods (GNRs) were synthesized using the seed-mediated growth method in fresh aqueous CTAB solution. In brief, for the preparation of gold seed solution, 0.25 mL of $HAuCl_4 \cdot 3H_2O$ (100 mM) and 7.5 mL of CTAB solution (95 mM) were mixed, and 0.06 mL of ice-cold sodium borohydride solution (100 mM) was added to the mixture under vigorous stirring. The solution mixture was allowed to react for 2 min and was stored at room temperature for 3 h. A growth solution containing 0.08 mL of silver nitrate solution (100 mM) was prepared by addition of 0.5 mL of $HAuCl_4 \cdot 3H_2O$ (100 mM) and 9.5 mL of CTAB solution (95 mM) under vigorous stirring. After the addition of 55 μL of ascorbic acid solution (100 mM), the color of the solution changed from yellow to colorless. Twelve microliters of gold seed solution was introduced into the growth solution and stirred for 10 s. The product solution was maintained for 24 h at room temperature without disturbing, and its color changed from colorless to reddish-brown as time went on. The GNRs solutions were centrifuged twice at 15 000 rpm for 30 min

to remove excess CTAB molecules and were then redispersed in 4 mL of deionized water.

Subsequently, 3 mL of prepared GNR solution (0.73 mg of Au/mL) and 20 mg of thiol-functionalized dextran were vigorously mixed for 24 h at room temperature. The mixture containing dextran-coated nanorods was centrifuged at 15000 rpm for 30 min to remove unbound dextran molecules and was then resuspended in 3 mL of deionized water.

Synthesis of PEGylated Gold Nanorods (PEG-GNRs). GNRs were coated with thiolated PEG as a stabilizer to produce PEGylated gold nanorods (PEG-GNRs). For the synthesis, 20 mg of mPEG-SH (M_w 2K, 5K, and 10K, respectively) was added to 3 mL of GNR solution (0.73 mg of Au/mL) and vigorously mixed for 24 h at room temperature. Finally, the mixture was centrifuged at 15000 rpm for 30 min to remove unbounded PEG molecules and resuspended in 3 mL of deionized water.

Cytotoxicity Study. The cell viability was evaluated using a WST-1 cell proliferation assay Takara (Japan) based on mitochondrial oxidation. RAW 264.7 cells, macrophage cell line, obtained from the American Tissue Type Culture (ATCC, USA) were seeded into a 96-microwell plate at a density of 2.0×10^4 cells/well and were incubated at 37 °C in a humidified atmosphere with 5% CO_2. After 24 h incubation, RAW 264.7 cells were treated with various concentrations of DEX-GNRs and PEGylated GNRs (PEG2K-GNRs, PEG5K-GNRs and PEG10K-GNRs at $4.0 \times 10^{-9} \sim 4.0 \times 10^{-3}$ mg of Au/mL) and then incubated for 24 h at 37 °C. The cells were washed with 100 μL of phosphate buffered saline (PBS, pH 7.4, 1 mM), and 100 μL of fresh media was added. Subsequently, the cells were treated with 10 μL of WST-1 solution and incubated for an additional 2 h. The cells were evaluated using an enzyme-linked immunosorbent assay (ELISA, Spectra MAX 340, Molecular devices, USA) at an absorbance wavelength range of 450−690 nm. All experiments were performed in triplicate. Cell viability was determined from the ratio of treated cells to nontreated control cells.

Cellular Affinity Test. RAW 264.7 cells (5.0×10^5 cells/well in a six-well plate) were incubated with 4.0 μg/mL of DEX-GNRs and PEGylated gold nanorods prepared using different molecular weights of PEG (PEG2K-GNRs, PEG5K-GNRs and PEG10K-GNRs) at 37 °C for 24 h. After the six-well plates were rinsed with PBS (pH 7.4, 1 mM), the cells were detached using a cell scrapper. The cellular uptakes of DEX-GNRs and PEG-GNRs were finally quantified via measurement of the Au concentration from gathered cells using ICP-AES. Percent (%) cell uptake representing the cellular binding affinity was calculated as the Au concentration localized in the cell/Au concentration of treated nanoparticles.

Intracellular Transmission Electron Microscopy (TEM) Image. For cellular TEM sample preparation, RAW 264.7 cells (5.0×10^5 cells/well) were seeded onto six-well plates for 24 h. The cells were then rinsed with PBS (pH 7.4, 1 mM), and DEX-GNRs and PEG-GNRs (PEG2K-GNRs, PEG5K-GNRs and PEG10K-GNRs) (4.0 μg of Au/mL) were added to Dulbecco's modified eagle medium (DMEM, Gibco, Invitrogen, USA). After incubation for 24 h at 37 °C, the cells with DEX-GNRs and PEG-GNRs were detached, centrifuged, and washed three times with blocking buffer (0.03% bovine serum albumin, 0.01% NaN_3 in PBS). The samples were then fixed with 2% glutaraldehyde-paraformaldehyde in 0.1 M PBS (pH 7.4) for 2 h and washed three times for 30 min in 0.1 M PBS (pH 7.4, 1 mM). They were post fixed with 1% OsO_4 (Osmium tetroxide) dissolved in 0.1 M PBS (pH 7.4) for 2 h, dehydrated in an ascending gradual series (50, 60, 70, 80, 90, 95, and 100%) of ethanol and infiltrated with propylene oxide. Specimens were embedded using the Poly/Bed 812 kit (Polysciences, USA). After being embedding in pure fresh resin at 60 °C in an electron microscope oven (TD-700, DOSAKA, Japan) for 24 h, 35-nm-thick sections were initially cut and stained with toluidine blue for observation under a light microscope (Olympus BX40, Japan). Seventy-nanometer-thick sections were double-stained with 7% uranyl acetate and lead citrate for contrast staining (20 min). The sections were then cut using a LEICA Ultracut UCT Ultramicrotome (Leica Microsystems, Austria). All of the samples were observed using transmission electron microscopy (TEM, JEM-1011, JEOL, Japan) at an acceleration voltage of 80 kV.

Dark-Field Microscopy. RAW 264.7 cells (5.0×10^5 cells/well) were seeded onto six-well plates and incubated at for 24 h 37 °C. Prepared DEX-GNRs and PEG-GNRs (PEG2K-GNRs, PEG5K-GNRs, and PEG10K-GNRs) (20 μg of Au/mL) were added to Dulbecco's modified eagle medium (DMEM, Gibco, Invitrogen, USA). After incubation for 24 h at 37 °C, the cells with DEX-GNRs and PEG-GNRs were detached, centrifuged, and washed three times with PBS (pH 7.4, 1 mM). To observe the optical absorption of the gold nanorods in the cells, the light scattering images were recorded using an inverted microscope (Olympus BX51) with a highly numerical dark-field condenser (U-DCW, Olympus), which delivers a very narrow beam of white light from a tungsten lamp to the surface of the sample. Immersion oil (nd: 1.516, Olympus) was used to narrow the gap between the condenser and the glass slide and to balance the refractive index. The dark-field pictures were captured using an Olympus CCD camera.

Photothermal Ablation Study. RAW 264.7 cells (2.0×10^4 cells/well) were split into the wells of a 96-well plate and allowed to grow for 24 h. The cells were then treated with 100 μL (4.0 μg/mL) of DEX-GNRs and PEG-GNRs (PEG2K-GNRs, PEG5K-GNRs and PEG10K-GNRs) at 37 °C for 24 h. For uptake inhibition assays, the cells were incubated with 100 μL (0.4 mg/mL) of dextran in media without FBS at 37 °C for 20 min. And then, we treated with 100 μL (4.0 μg/mL) of DEX-GNRs at 37 °C for 24 h. The cells were then rinsed with PBS (pH 7.4, 1 mM), and 100 μL of DMEM was added into each well. For the NIR laser irradiation experiment, the cells were exposed to a NIR coherent diode laser for 5 min at two different powers (1 and 2.5 W/cm^2). Then the samples were washed with PBS and further incubated for 24 h. The distribution of the live cells was observed using an optical system microscope (Olympus BX51, Japan) after cellular staining with 100 μL of calcein AM (1 μM, Molecular Probes, USA) for 30 min. For quantification of the therapeutic efficacies, the cells were treated with 10 μL of WST-1 and incubated for an additional 2 h. The cells were evaluated using an enzyme-linked immunosorbent assay (ELISA, Spectra MAX 340, Molecular devices, USA) at an absorbance wavelength range of 450−690 nm.

Characterization. The morphologies, sizes and zeta potentials of the synthesized GNRs, PEG-GNRs and DEX-GNRs were analyzed using high resolution transmission electron microscopy (HR-TEM, JEM-2100 LAB6, JEOL Ltd., Japan) and laser scattering (ELS-Z, Otsuka electronics, Japan). In addition, their absorption spectra were determined by a UV−vis spectrometer (Optizen 2120UV, MECASYS, KOREA). The characteristic bands of thiolated dextran, DEX-GNRs and PEG-GNRs were evaluated using Fourier-transform infrared spectroscopy (FR-IR, Varian, Excalibur, USA). The amounts of Au incorporated into the DEX-GNRs and PEG-GNRs were quantified using inductively coupled plasma atomic emission spectrometry (ICP-AES, Thermo electron corporation, USA).

Results and Discussion

As a strong NIR photothermal agent, GNRs were synthesized using a seed-mediated growth method.[14] Sodium borohydride was used as a strong reduction agent to form the gold nucleuses from reduction of Au^{3+} ions. The gold nanoparticle seeds were subsequently coated with CTAB because the electron-dense Au

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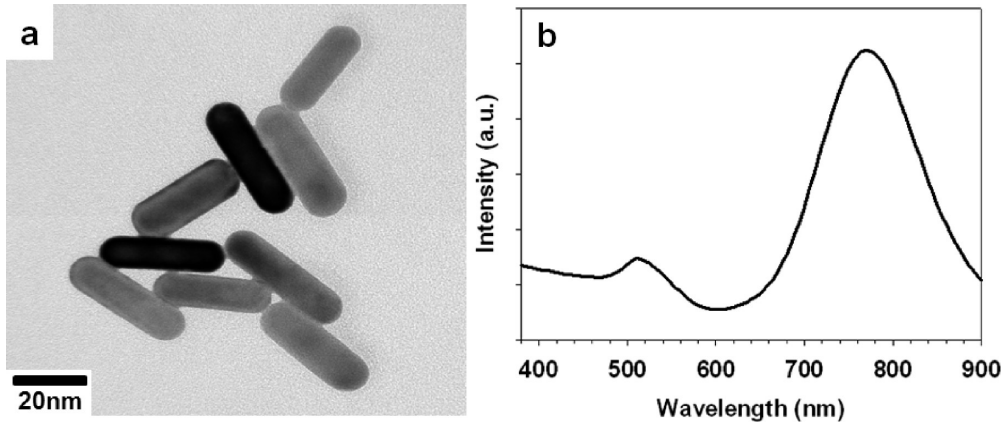

Figure 2. (a) TEM image and (b) UV−vis absorption spectrum of GNRs.

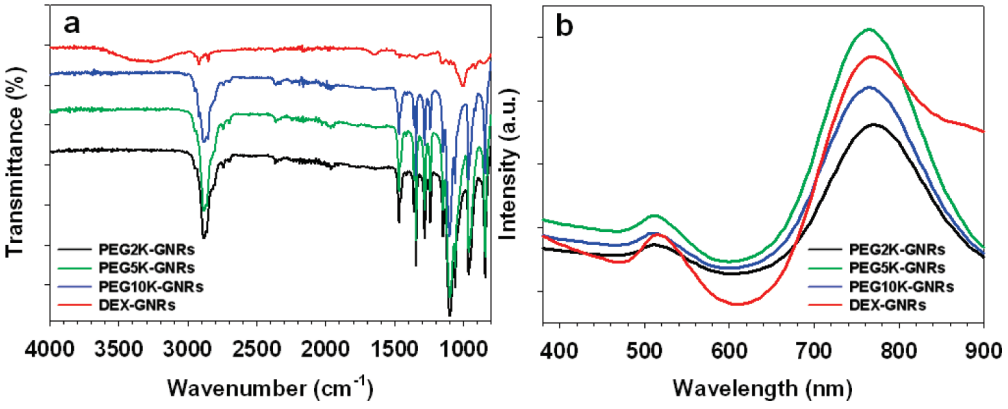

Figure 3. (a) FT-IR spectra and (b) optical absorption spectra of PEGylated GNRs (PEG2K-GNRs, PEG5K-GNRs, and PEG10K-GNRs) and DEX-GNRs.

atoms interact with the positively charged trimethylammonium headgroup of CTAB. The added Ag ions from silver nitrate were deposited onto the {110} surface of the gold nanoparticle seeds, leading to rod-shaped nanostructures.[15] The rod-like shape of the synthesized gold nanostructures was confirmed via high resolution TEM imaging, and their aspect ratio (longitudinal length/transverse length) was 3.6 (Figure 2a). Moreover, the absorption peaks of GNRs were measured at 510 and 760 nm due to the oscillation along the transverse and longitudinal axes, respectively (Figure 2b). These results demonstrate that the synthesized GNRs retained the potential for photothermal ablation of target cells due to strong absorption in the NIR region.

Since CTAB as a stabilizer exhibits a cytotoxic phenomenon and cellular function interference due to its strong electric potential, the surfaces of the gold nanoparticles should be modified with inert ligands like as dextran in order to enhance the biocompatibility of the GNRs. Furthermore, dextran can be used as an inflammatory macrophage targeting moiety due to the existence of scavenger receptors on the macrophage surfaces.[9] To formulate the thiolated dextran-coated GNRs (DEX-GNRs), we first prepared thiolated dextran using mercaptoproionic acid and dicyclohexylcarbodiimide as a modifying reagent due to the strong affinity between the gold and the thiol group. The existence of a thiol group in the functionalized dextran was determined using FT-IR spectrum and Ellman's reagent in a thiol group assay. The characteristic band of thiolated dextran was confirmed at 1652 cm^{-1} (ester bond). Also, the optical absorption of thiolated dextran with Ellman's reagent at 420 nm indicates the existence of a thiol group in the synthesized polymer, which demonstrates successful conjugation of the hydroxyl groups of dextran with the carboxyl group of mercaptoproionic acid. We then calculated the concentration of -SH groups in thiolated dextran based on molar absorptivity which is 4.67 M. Thus, dextran-coated GNRs (DEX-GNRs) were formulated through the ligand exchange process between CTAB and thiolated dextran. The FT-IR spectrum of DEX-GNRs illustrated the thiolated dextran peak at 1652 cm^{-1} (Figure 3a), and its optical absorption peaks were observed at 510 and 765 nm, similar to those of CTAB-coated GNRs (Figure 3b).

To assess the cellular uptake efficiency of DEX-GNRs, a control experiment with PEGylated GNRs was also prepared by replacing CTAB with thiolated PEG using different molecular weights of PEG (2K, 5K, and 10K). In Figure 3a, the FT-IR spectra of three samples of PEGylated GNRs (PEG2K-GNRs, PEG5K-GNRs, and PEG10K-GNRs) were observed at 2877 cm^{-1} (methyl and ethyl group from the PEG backbone), and their absorption peaks at 510 and 765 nm were analyzed (Figure 3b).

After the preparation of DEX-GNRs and PEG-GNRs, we investigated the colloidal stability of DEX-GNRs and PEG-GNRs, which is demonstrated excellent deionized water solubility for over 30 days without any aggregation as shown in Figure 4a. Then the morphological deformation of DEX-GNRs and PEG-GNRs were not observed via TEM (Figure 4b).

Moreover, the zeta potentials of ligand-exchanged GNRs in deionized water were changed by -2.6 ± 0.3 mV (DEX-GNRs),

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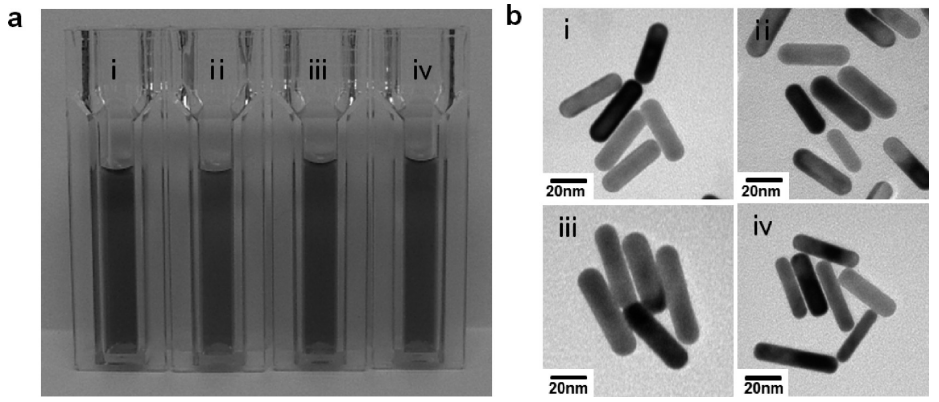

Figure 4. (a) Photographs for the colloidal stability against the prepared nanoparticles in deionized water after 30 days from the preparation and (b) their TEM images of (i) PEG2K-GNRs, (ii) PEG5K-GNRs, (iii) PEG10K-GNRs, and (iv) DEX-GNRs.

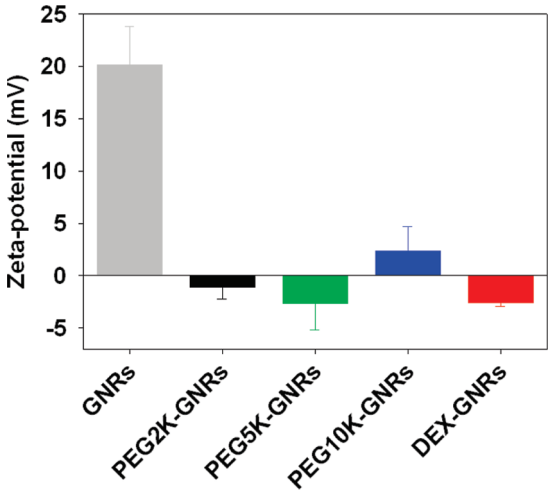

Figure 5. Zeta-potential analysis for CTAB-coated GNRs, PEGylated GNRs (PEG2K-GNRs, PEG5K-GNRs, and PEG10K-GNRs), and DEX-GNRs.

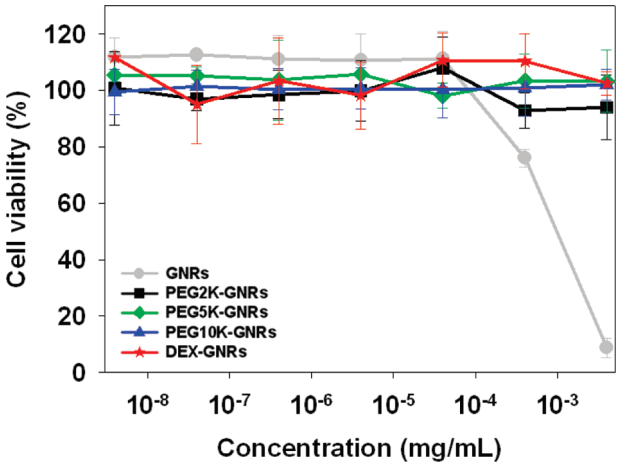

Figure 6. Growth-inhibition assay for RAW 264.7 cells treated with CTAB-coated GNRs, PEG-GNRs (PEG2K-GNRs, PEG5K-GNRs, and PEG10K-GNRs), and DEX-GNRs.

-1.1 ± 1.2 mV (PEG2K-GNRs), -2.6 ± 2.5 mV (PEG5K-GNRs), and 2.4 ± 2.3 mV (PEG10K-GNRs) from the highly positive charge (20.1 ± 3.7 mV) of CTAB-coated GNRs (Figure 5). These results indicate that the ligand-exchanged GNRs based on biocompatible polymers were successfully prepared using the ligand exchange process.

We next estimated the cell viability of DEX-GNRs on the macrophages (RAW 264.7 cells) using the WST-1 assay, which is highly recommended as a cell proliferation reagent because the additional solubilization step is not essential, in contrast to the MTT assay. Compared to CTAB-coated GNRs, DEX-GNRs and PEGylated GNRs (PEG2K-GNRs, PEG5K-GNRs, and PEG10K-GNRs) retained a much lower cytotoxicity, particularly at a high nanoparticle concentration (4.0×10^{-3} mg of Au/mL; Figure 6). These nontoxic surface-modified GNRs were then investigated for their targeting efficiency with macrophages.

To evaluate the cellular affinity of the prepared gold nanoplatforms, the binding and uptake efficiency for RAW 264.7 cells were investigated using microscopic images. In Figure 7a, the scattered light from DEX-GNRs and PEGylated GNRs (PEG2K-GNRs, PEG5K-GNRs, and PEG10K-GNRs) internalized within RAW 264.7 cells were observed as an orange color. In particular, intensely scattered light was observed from RAW 264.7 cells treated with DEX-GNRs compared to those of PEG-GNRs due to the strong interaction between dextran and the scavenger receptor of the macrophages. Furthermore, this superior affinity of DEX-GNRs for macrophages was verified using the cellular TEM image. A large amount of DEX-GNRs was specifically internalized into the cytoplasm of RAW 264.7 cells (Figure 7b) compared with the amount of PEGylated GNRs. In addition, the binding affinity of DEX-GNRs for macrophages was also quantified by measuring the Au amounts from GNRs entrapped by RAW 264.7 cells using ICP-AES after incubation of macrophages (5×10^5 cells) with DEX-GNRs (4.0×10^{-3} mg of Au/mL). In Figure 8, DEX-GNRs exhibited an approximately 2.5-fold higher cell uptake capability ($54.7 \pm 0.5\%$) compared to that of the PEGylated GNRs (PEG2K-GNRs: $20.2 \pm 0.03\%$, PEG5K-GNRs: $22.0 \pm 0.10\%$, and PEG10K-GNRs: $22.8 \pm 0.50\%$). These results can be explained by the fact that cellular uptake kinetics are affected by the characteristics of surface molecules.[16]

To investigate the photothermal characteristics of DEX-GNRs, we measured the temperature changes of solutions under NIR laser irradiation (Figure 9). Both DEX-GNRs and PEGylated GNRs (PEG2K-GNRs, PEG5K-GNRs, and PEG10K-GNRs) under NIR light irradiation generated similar and effective photothermal heat in solution, with temperature increases of 24 °C to 48−52 °C, which is sufficient to induce cell damage. In contrast, in the absence of GNRs, the temperature change was insignificant (24 to 27 °C).

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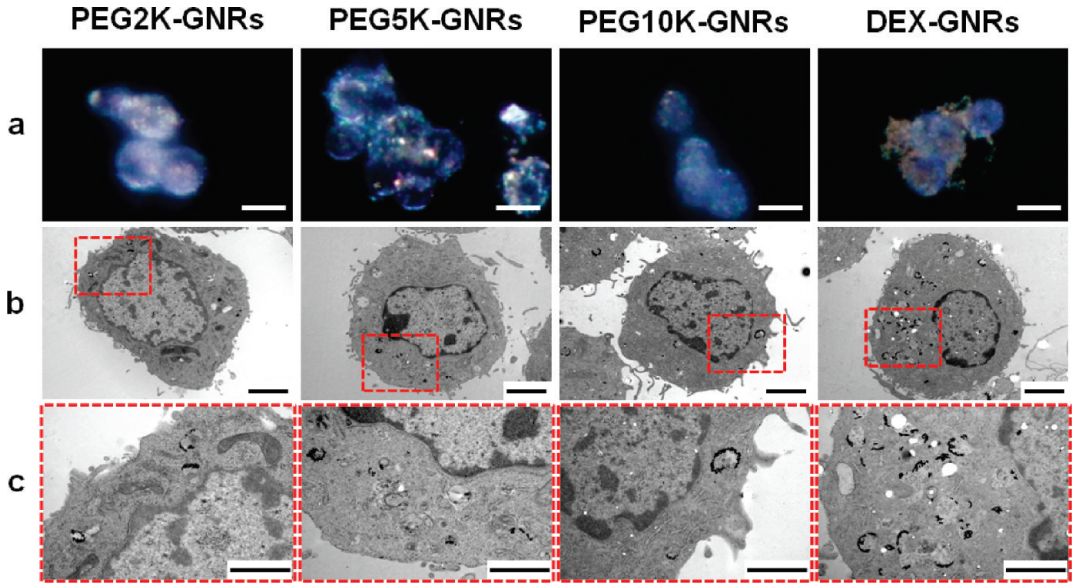

Figure 7. (a) Dark-field microscopic images of RAW 264.7 cells treated with PEGylated GNRs (PEG2K-GNRs, PEG5K-GNRs and PEG10K-GNRs) and DEX-GNRs (scale bar: 200 μm) and (b) their cellular TEM images. Scale bar: 2 μm. (c) High magnification TEM images of (b). Scale bar: 1 μm.

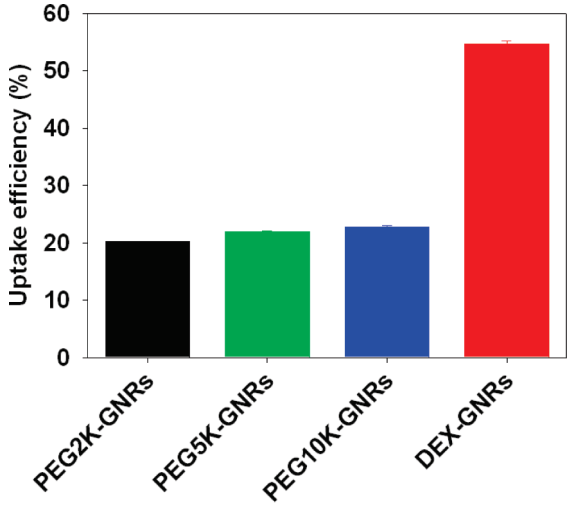

Figure 8. Cellular uptake efficiencies of PEG-GNRs and DEX-GNRs for RAW 264.7 cells (error bars represent the standard deviations).

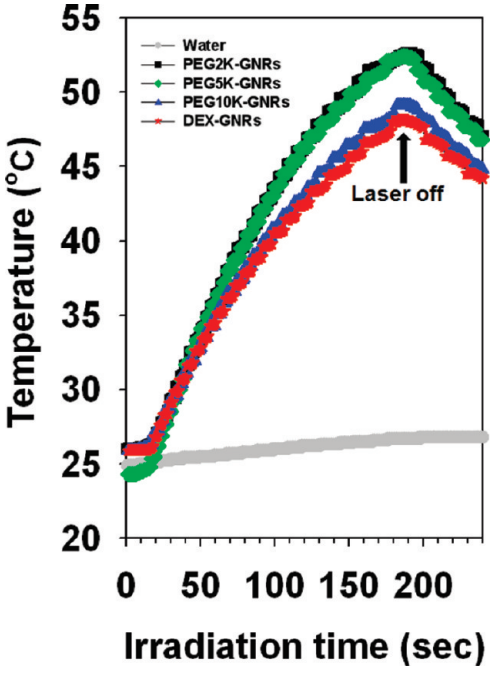

Figure 9. Temperature profiles for PEG-GNRs solutions and DEX-GNRs solutions (70 μg of Au/mL) with an NIR laser irradiation (808 nm) at a power density of 4.4 W/cm^2 for 3 min.

For assessment of the photothermal ablation potentials for macrophages, RAW 264.7 cells (2×10^4 cells/well) were incubated with DEX-GNRs and PEGylated GNRs (PEG2K-GNRs, PEG5K-GNRs, and PEG10K-GNRs) and with 4.0×10^{-3} mg of Au/mL for 24 h in 96-well culture plates and were washed to remove the unreacted nanoparticles. An NIR coherent diode laser (808 nm) was used to irradiate each well for 5 min with a different laser power density (1.0 and 2.5 w/cm^2). Calcein AM staining was conducted to classify the live/dead cells, and a strong dark spot (unstained dead cells) was revealed by the combination of DEX-GNRs and NIR laser at both powers of 1 and 2.5 W/cm^2 (Figure 10a). In contrast, the cellular damage from PEGylated GNRs (PEG2K-GNRs, PEG5K-GNRs, and PEG10K-GNRs) was insignificant, even at a power of 2.5 W/cm^2, because of the low cellular uptake efficiency. In the quantification analysis, PEGylated GNRs groups did not change the cell viabilities of RAW 264.7 cells; moreover, DEX-GNRs demonstrated a cytotoxic effect with low cell viability (7.5 $\pm$ 1.6%) under NIR laser irradiation (both 1.0 and 2.5 W/cm^2; Figure 10b). At temperatures greater than 40 °C, irreversible damage was induced in the cells, resulting from protein denaturation due to changes in either enzyme complexes for DNA synthesis and repair or multimolecular structures of the cytoskeleton and membranes.[17] To confirm the receptor specificity of DEX-GNRs for macrophages, the inhibition test was performed using free dextran. Dextran molecules as an inhibitor were treated prior to the addition of DEX-GNRs into the wells. After the irradiation

(17) van der Zee, J. *Ann. Oncol.* **2002**, *13*(8), 1173.

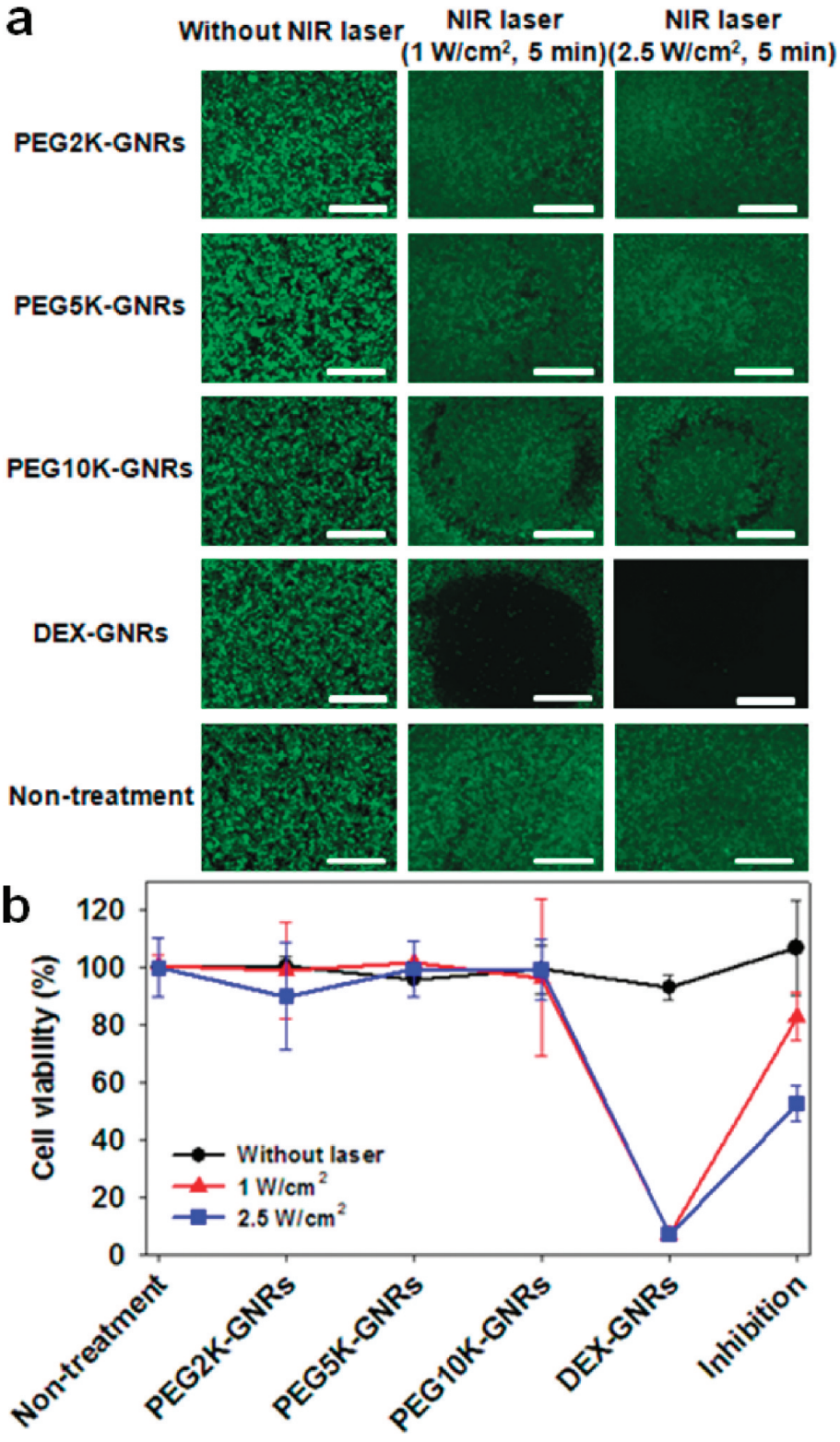

Figure 10. (a) Fluorescence microscopic images of macrophages treated with GNR solutions and DEX-GNRs after NIR laser irradiation. Scale bar: 1.0 mm. (b) Cell viability of RAW 264.7 cells after NIR laser irradiation, power density: 0 W cm^{-2} (circle and black line), 1 W cm^{-2} (triangle and red line), and 2.5 W cm^{-2} (rectangle and blue line).

with the NIR laser (808 nm), the cell viabilities of RAW 264.7 cells treated with DEX-GNRs increased by 83.0 ± 8.5% (1.0 W/cm^2) and 52.7 ± 6.2% (2.5 W/cm^2) compared with those of the noninhibited experimental conditions. The cellular uptake of GNRs was diminished due to pretreatment with free dextran molecules that increased the cell viability. Therefore, these results demonstrate that DEX-GNRs are delivered to macrophages via scavenger receptors and receptor-mediated endocytosis. Furthermore, the macrophage-specific DEX-GNRs can produce a remarkable cellular destruction effect due to the photothermal heat with even a weak NIR light source, the use of which can minimize the side effects to normal tissue.

Conclusion

We successfully synthesized nontoxic dextran-coated gold nanorods (DEX-GNRs) for efficient delivery into macrophages. In comparison to PEGylated GNRs, DEX-GNRs revealed significant photothermal ablation efficacy at a low dose and low laser power, and this result supports its use in potential capabilities for the treatment of inflammatory macrophages related to diseases, although more work is necessary to advance this nanoplatform for further clinical applications.

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