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Near-Infrared Fluorescent Endoscopic Image-Guided Photothermal Ablation Therapy of Colorectal Cancer Using Dual-Modal Gold Nanorods Targeting Tumor-Infiltrating Innate Immune Cells in a Transgenic *TS4 CRE/APC^{lox} 468* Mouse Model

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Abstract

Colorectal cancer (CRC) is diagnosed with colonoscopy and treated with focal therapies. CRC is a good candidate for nanoparticle-mediated photothermal ablation (PTA) therapy. Herein, we developed a near-infrared fluorescent (NIRF) endoscopic image-guided PTA approach using a nanoparticle capable of simultaneously diagnosing and treating CRC. Dual-modal NIR heating and fluorescent gold nanorods (dual-modal GNRs) were synthesized by conjugation of GNRs to an NIRF probe. To validate the translational potential of our approach, a well-characterized transgenic *TS4 CRE/APC^{lox} 468* colon cancer mouse model was used to carry out NIRF image-guided PTA using our dual-modal GNRs under clinically relevant conditions. Intravenously infused dual-modal GNRs were effectively targeted at colon polyps by immunogenic capturing of the GNRs within tumor-infiltrating innate immune cells. NIRF endoscopic image-guided PTA using the GNRs permitted successful detection and ablation of inflammatory colon polyps. NIRF endoscopy image-guided PTA using dual-modal GNRs can be utilized for diagnosis and treatment of CRC and various inflammatory diseases.

Graphical Abstract

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Supporting Information

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Digital and in vivo IR thermal camera images of a GNR-targeted polyp irradiated with an NIR laser ([PDF](#))

The authors declare no competing financial interest.

Keywords

colon cancer; endoscopic image-guided therapy; nanomedicine; interventional oncology; photothermal therapy

1. INTRODUCTION

Colorectal cancer (CRC) is the 3rd most common malignancy and 4th most common cause of cancer mortality worldwide.¹ CRC is also the 3rd most common cancer diagnosed in both men and women in the United States, despite important advances in detection, surgery, and chemotherapy.^{1,2} More than 1.4 million new cases of CRC are diagnosed worldwide each year.¹ It was estimated to have caused roughly 50,630 deaths in 2018.² The most common treatment options for CRC are surgery, radiation therapy, and systemic chemotherapy but the therapeutic efficacy of these approaches is limited and severe side effects are common.

Colonoscopy plays an important role in the localization of malignant lesions and subsequent identification at the time of surgery. It is widely accepted that early detection of a cancerous disease permits a broader spectrum of treatment options and improved outcomes.³⁻⁵ The use of high-definition white light endoscopy, the gold standard, has contributed to improved detection of early polypoid lesions. However, as white light detection of CRC is based upon structural changes, not upon functional criteria, it is still difficult to detect intraepithelial dysplasia and discriminate pseudo-polyps from polypoid dysplasia. Near-infrared fluorescent (NIRF) endoscopy with fluorescent probes may be a superior choice for the localization of CRC. Endoscopically detected pedunculated lesions are typically removed by using standard snare polypectomy, mechanical or electrocautery resection.⁶ As an alternative treatment, endoscopic laser therapy can provide relief of obstruction and bleeding in patients.⁷ Neodymium yttrium argon garnet (Nd:YAG) lasers (1024 nm) have been used to treat large, sessile, and benign polyps.⁷ However, nonspecific heating by the laser can result in coagulative necrosis of tissue with subsequent tissue sloughing and immediate vaporization of normal tissues. There are multiple potential complications during these laser ablation procedures including perirectal abscess formation, anal stenosis, and perforation.^{8,9} Perforations in the proximal colon result in spoilage of the free peritoneal space and often require surgical intervention.¹⁰ Recent studies showed no difference in life expectancy upon

laser treatment of these lesions when compared to surgical resection.^{11,12} The greatest challenge for these therapeutic modalities is to treat only localized tumors while preserving adjacent tissues to minimize potential damage to normal tissues.^{13,14} Nanoparticle-mediated NIR photothermal ablation (PTA) therapy offers considerable promise for the detection and targeted treatment of inflammatory CRC, displaying robust pro-inflammatory innate immune cell infiltration and increased expression of proinflammatory cytokines.^{15–17} Recruited inflammatory macrophages, having an intrinsic disease-homing property, can be utilized to target nanoparticle delivery to CRC regions.^{18,19} Nanoparticles targeted at macrophages associated with proinflammatory substances have been utilized to visualize and treat various inflammatory diseases.^{20,21}

Here, we report NIRF endoscopic PTA of CRC combined with immunogenic targeted NIRF gold nanorods (GNRs) for in situ identification and ablation of dysplastic lesions (Scheme 1). GNRs are considered a promising agent for photothermal therapy (PTT), given that these generate heat more rapidly compared to spherical gold nanoparticles, and GNRs can be readily synthesized with a tunable absorption wavelength.²² In our study, GNRs were tuned for efficient NIR photothermal heating and conjugated with NIR fluorophores (Cy 5.5) for CRC detection and PTA with a multichannel NIRF endoscope. To validate potential clinical translation, a clinically relevant transgenic *TS4 CRE/APC^{lox}*⁴⁶⁸ colon cancer mouse model was used to evaluate the efficacy of nanoparticle-mediated NIR endoscopic image-guided PTA. The selective accumulation of GNRs within inflammatory macrophages in CRC was characterized with in situ NIRF endoscopic imaging using cathepsin-activatable probes and immune fluorescent histology analysis. Finally, NIRF endoscopic image-guided PTA using the GNRs was able to target dysplastic lesions in the transgenic colon cancer mouse model.

2. MATERIALS AND METHODS

2.1. Materials.

HAuCl₄·3H₂O (99.9%), L-ascorbic acid (AA; 99%), NaBH₄ (99%), AgNO₃ (99%), and cetyltrimethylammonium bromide (CTAB; 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Amine–PEG–thiol (*M_w*: 5 kDa) was obtained from Laysan Bio (Arab, AL, USA). Cyanine5.5 *N*-hydroxysuccinimide ester (Cy5.5-NHS) was purchased from Lumiprobe (FL, USA). ProSense 750 fluorescent activatable sensor technology was purchased from PerkinElmer (MA, USA).

2.2. Preparation of Gold Nanoseeds.

An aqueous 0.01 M solution of HAuCl₄·3H₂O (0.25 mL) was added to a 0.10 M CTAB solution (7.5 mL). The solutions were carefully mixed through inversion. The color of the solution changed to bright brown-yellow. A cold 0.01 M NaBH₄ solution (0.06 mL) was added to the solution and then mixed for 2 min. The color of the mixture was immediately changed to a pale brown-yellow color and was further reacted for 2 h in a water bath maintained at 25 °C.

2.3. Seed-Mediated Preparation of GNR.

CTAB (0.10 M, 4.75 mL), 0.01 M $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (0.20 mL), and 0.01 M AgNO_3 solution (0.01–0.10 mL) were added in a test tube sequentially and mixed gently. The color of the mixture changed to bright brown-yellow. Then, 0.10 M AA (0.032 mL) was added to the mixture. Finally, after adding the seed solution (0.01–0.05 mL), the mixture was gently mixed for 10 s, and the solution was left at room temperature for 3 h. To synthesize surface-modified GNRs with polyethylene glycols (PEGs), 2 mM amine-PEG-thiol solution (100 μL) was added to the 0.02 M GNR solution (10 mL). The mixture was stirred for 3 h at room temperature. The PEG-modified GNRs were centrifuged for 10 min at 8500 rpm. After removing the supernatant, the PEG-modified GNRs were re-dispersed in PBS solution (5 mL).

2.4. NIR Fluorophore Labeling on the GNR.

The PEG-modified GNRs (10 mg) were re-dispersed in 0.1 M sodium bicarbonate buffer (1 mL, pH 8.3), and Cy5.5-NHS (0.03 mg) was added. The mixture was left to react for 12 h at room temperature. To remove unconjugated Cy 5.5, dialysis was performed at 10 mM PBS for 24 h.

2.5. Characterization of the Dual-Modal GNR.

The absorption characteristics of the GNR were measured using a UV-vis spectrophotometer (cytation 3, BioTek, USA). The form of synthesized GNR was observed through transmission electron microscopy (TEM, Hitachi H-8000, Hitachi, Tokyo, Japan). To confirm the fluorescence of NIR fluorophore-labeled GNRs, samples were imaged with a fluorescent OV100 imaging system (Olympus, Tokyo, Japan). The fluorescent image was processed and analyzed using Image-Pro Plus Software (Olympus, Tokyo, Japan).

2.6. Depth-Dependent Photothermal Heating.

NIR diode-laser (808 nm, BWF5, B&W Tek, Inc., DE, USA) connected to fiber optics was used to confirm the photothermal characteristics of the synthesized GNR. The GNR was re-dispersed to 1% agar phantom and then transferred to a plastic cuvette (Malvern, Herrenberg, Germany). Each sample was characterized under different-strength laser conditions. The thermal properties of GNR by NIR laser irradiation were investigated through an IR camera (ICI7320P, Beaumont, TX, USA). In this experiment, 808 nm laser at 1 W/cm^2 was used, and irradiation was performed at a distance of 1 cm from the sample. The laser was irradiated for 60 s, and the laser power was adjusted by the distance between the optical fiber and the sample.

2.7. In Vivo Real-Time Endoscopic Observation of Targeted Dual-Modal GNR on Polyps.

A transgenic *TS4 CRE/APC^{lox} 468* colon cancer mouse model was implemented for in vivo studies, typically producing 5–6 colon polyps at the age of 5–6 months.^{23,24} All procedures involving experimental animals were performed by protocols approved by the Northwestern University Institutional Animal Care and Use Committee. A cathepsin active probe (2 nmol, ProSense 750) was injected into the mouse 16–24 h before endoscopy. For smooth endoscope insertion, PBS was injected before the insertion of the endoscope in the mouse

colon. A fiberscope (BFXP60, Olympus, Tokyo, Japan) was used in this experiment. This dual laser fluorescence endoscope produces two distinctive excitation lights (680 and 750 nm) with a little cross-talk between the channels.²⁵ The images (300 × 300 pixels) obtained through endoscopy were analyzed and converted to video via NIH ImageJ software. The mean fluorescence intensity was calculated using the NIH ImageJ software after selecting three regions of interest (30 × 30 pixels) in the obtained images. The final calculated fluorescence intensity was plotted using GraphPad Prism5 software (GraphPad Software Inc, San Diego, CA, USA).²⁶

2.8. NIRF Endoscopy Imaging and Selective Dual-Modal GNR-Mediated PTA.

Previously reported NIRF endoscopy was used for this study.²⁵ Two laser light sources (680 and 750 nm) with filters to avoid the cross-talk were fitted to the Olympus BF-XP60 fiberscope. The fiber endoscope has a 90° field of view with a 2–50 mm local depth. The total length of the endoscope was 600 mm, and its outer diameter was 2.8 mm. The endoscope was equipped with a 1.2 mm instrument channel and was able to bend the end tip 180° upwards and 130° downwards. The fluorescent and reflectance light signals were processed by a high-sensitivity CCD camera through a splitter. The emitted fluorescent and the transmitted signal was recorded at 10 frames per second.^{16,27} The efficacy of NIRF endoscopy-guided PTT with dual-modal GNRs for the ablation of polyps was evaluated in *TS4 CRE/APC^{lox} 468* mouse models. Dual-modal GNRs (Ex = 680 nm) and a ProSense 750 (Ex = 750 nm) cathepsin activity probe for polyp detection were injected by the retro-orbital route. After 24 h, the mice were subjected to endoscopy with a 680 nm laser to detect the focal distribution of dual-modal GNRs. Polyps were detected using a 750 nm laser. Images were collected and evaluated for focal increases of the emissions of the probes. Then, the NIR laser (power, 1 W/cm² for 10 s) was irradiated to the polyps. The fine structure of the lesions before and after the PTT, and the focal distribution of dual-modal GNRs were verified with intravital microscopy and reflectance fluorescence.

2.9. Histopathology Assessments.

Colon polyps with mouse intestine were fixed in 10% formalin solution for 24 h. The tissue specimens were sliced at 1 mm intervals and then sectioned to a thickness of 5 μm. H&E and TUNEL staining were used to determine necrotic/apoptotic tumor tissues. The tissue was analyzed through a TissueFAXS microscope (TissueGnostics GmbH, Vienna, Austria). For immunofluorescence studies, sequential 5 μm thick optimal cutting temperature (OCT) frozen tissue sections were stained with anti -F4/80 and Gr1 antibodies and counterstained with DAPI. The images were collected with the TissueFAXS microscope.

3. RESULTS AND DISCUSSION

3.1. Synthesis of Dual-Modal GNRs.

GNRs were synthesized through a two-step synthesis process. After synthesizing GNR capable of absorbing light in the NIR region, fluorescent molecules were conjugated to the surface of the GNR. First, the amount of AgNO₃ in the synthesis process was adjusted to synthesize the GNR of appropriate size to absorb NIR wavelength light. As the amount of AgNO₃ increased, the aspect ratio of the GNR also increased, and the absorption wavelength

shifted to the NIR wavelength (Figure 1a). In this study, the GNR with the size of 12.7 ± 3.4 nm (width) and 47 ± 9.3 nm (length) having the highest absorption at an NIR wavelength of around 800 nm was selected and used for further in vitro and in vivo experiments (Figure 1b). The aspect ratio of the GNR was 3.7. In order to introduce a fluorescent probe to the surface of GNRs for optical imaging and reduce their toxicity, cytotoxic surface ligands (i.e., CTAB) of the GNR were replaced with biocompatible polyethyleneglycol (PEG, 5 kDa) molecules bi-functionalized with thiol and amine groups. The terminal amine group of PEG can improve the uptake by macrophages in vivo by making GNR surfaces positively charged.^{28,29} After the surface modification with PEG, it was determined that the GNRs contained ~20 000 polymers per particle according to the N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) assay.³⁰ An NIR probe (Cy 5.5; Cyto 680) was conjugated to the amine group at the end of the PEG molecule (Figure 1c). When examined with an in vivo imaging station (Olympus Corp., Tokyo, Japan), the Cy5.5-labeled GNRs showed an excellent fluorescent signal as anticipated (Figure 1d). This result implies that the Cy5.5-GNRs are suitable for optical fluorescence imaging using NIRF endoscopy. Henceforth, in this report, the GNRs conjugated with NIR fluorescent probes will be described as dual-modal GNRs.

3.2. Evaluation of the Photothermal Effects of Dual-Modal GNRs.

To confirm the photothermal effects of the as-synthesized dual-modal GNRs, the nanoparticles were embedded in an agarose gel and irradiated with an NIR laser to evaluate temperature changes (Figure 1e). The tissue penetration rate of the laser source can greatly affect the ablation efficacy during photothermal therapy.³¹ The NIR wavelength (808 nm) laser used in this study is known to have high tissue permeability.^{32,33} A nanoparticle-embedded agarose gel with a thickness of 1 cm was prepared and irradiated with an NIR laser at a distance of 1 cm from the agarose gel. Temperature changes of the gels were measured at different positions over time using a thermal camera while irradiating with the laser (Figure 1f). The temperature increase rate at 1 cm distance from the laser source demonstrated the fastest heating. All sites within 2 cm of where the dual-modal GNRs were embedded showed a tendency to increase with time when irradiated with the laser (Figure 1g). These results provided information about NIR laser power and distance-dependent spatial heating profiles between the laser source and lesions for effective PTA of epithelial and intraepithelial dysplasia.

3.3. NIRF Endoscopic Detection of Polyps Using Dual-Modal GNRs.

To perform an endoscopic image-guided PTA with dual modal GNRs in a colon cancer in vivo, a microendoscope device with a laser permitting dual excitation wavelengths was set up as described in refs.^{16,27} The tool channel of the BF-XP60 fiberscope was used to insert the fiber optic 808 nm NIR laser for photothermal therapy (Figure 2b). The tip of the fiberscope was guided to allow the laser to be placed on the tumor area. In this study, we used the transgenic *TS4 CRE/APC^{lox 468}* mouse model.²³ This transgenic mouse model faithfully develops only colon polyps. No polyps are detected in the small bowel, and the mice can live longer as they are not anemic as compared to adenomatous polyposis coli (APC) mice. To image colon polyps with NIRF endoscopy, an NIRF ProSense 750 probe, which is specifically activated by cathepsin enzymes in the tumor environment, was injected

into the animal model. As shown in Figure 2b (left), the tumor was imaged using the ProSense 750 probe. Then, the Cy5.5-labeled dual-modal GNRs were injected into the colon cancer mouse model. The accumulation of the injected dual-modal GNRs at the tumor site was confirmed by the co-localized fluorescence observed when compared to ProSense 750 NIRF endoscopy [Figure 2b (right)]. Macrophages, uptaking nanoparticles with innate phagocytotic properties, can serve as a “Trojan Horse” delivery vector of nano-medicine.^{20,34} Macrophages capturing nanoparticles have strong potential for cancer therapy because the macrophages can by-pass nearly impermeable tissue barriers to reach many areas in the body where common nanoparticles cannot reach.^{34–37} The recognition of these rod-shaped gold nanoparticles by the macrophage system is consistent with previous reports.^{34,38,39} To confirm the recognition of the dual-modal GNRs by macrophages and accumulation in a tumor site, the polyp tissue was extracted for immunofluorescent study after GNR injection. Green fluorescence for the dual-modal GNPs and red fluorescence for macrophages overlapped in the tumor tissue (Figure 2c). This result indicates that the dual-modal GNR-captured macrophages migrate to inflammatory colon cancer regions. Then, to examine the photothermal effect of dual-modal GNRs at the disease site, the GNRs were injected into the mice model via iv, and the small intestines with colon polyps from the mice were extracted to measure NIR photothermal heating. As shown in Figure 2d, the temperature of the tumor tissue was significantly increased compared to the surrounding tissue. GNR-mediated photothermal heating was also confirmed in vivo heating of the colon polyps during blood circulation (Supporting Information, Figure S1). These results indicate that the injected dual-modal GNRs are effectively targeted at tumors through tumor-infiltrating macrophages, thereby enabling tumor-specific PTA with NIRF endoscopic image guidance.

3.4. In Vivo Therapeutic Efficacy of NIRF Endoscopic Image-Guided PTA Therapy with Dual-Modal GNRs in a Transgenic *TS4 CRE/APC^{lox 468}* Colon Dysplasia Mouse Model.

Finally, we confirmed the PTA therapeutic effect of NIRF endoscopic image-guided PTA therapy with dual-modal GNRs in a transgenic *TS4 CRE/APC^{lox 468}* colon dysplasia mouse model. To induce a carcinogenic process in the colon, homologous recombination was performed to introduce loxP sites of the mouse *APC^{lox 468}* gene.^{40,41} These transgenic mice are an appropriate animal model for studying inflammation and colonic polyposis.

After injecting the GNRs into mice, the colorectal polyps detected by Cy5.5-GNRs were selectively irradiated by an NIR laser through an NIRF endoscopic guidance with a ProSense 750 probe, as shown in the real-time NIR endoscopic images (Figure 3). As Step 1, a polyp was detected with Cy5.5 GNRs. The detected polyp was then readily irradiated with an NIR laser equipped within an endoscope. After a single session of laser irradiation, the polyp was successfully ablated (Figure 3). After the procedure, the entire colon of the animal model was extracted and histologically stained to confirm the treatment of colon polyps by the photothermal effect. The polyp area showed significant cell death according to H&E and TUNEL staining (Figure 4a,b). The fluorescent immunohistochemistry of the treated tissue showed an interface area between normal tissues and the polyp, the latter co-localizing fluorescence from GNRs (red) and macrophages cells (green). The area with GNR-containing macrophage accumulation was selectively ablated after the NIR laser irradiation (Figure 4c). Neutrophil staining, one marker for inflammatory polyps, confirmed

the preferential GNR distribution in the polyp area and precise ablation of the polyp. This result implies that the targeted dual-modal GNRs delivered via immunogenic macrophages can be used to effectively treat colon cancers via NIRF endoscopy-guided PTA with dual-modal GNRs.

4. CONCLUSIONS

Nanoparticle-mediated PTA has rapidly advanced from concept to clinical trials for the PTA treatment of various cancers. PTA combined with imaging tools to guide therapeutics and monitor the success of treatment will offer great potential for increasing therapeutic efficacy while decreasing off-target delivery to healthy tissue. In this study, NIRF endoscopic image-guided PTA was performed with NIR fluorophore-labeled GNRs for effective CRC treatment. The dual-modal GNRs, capable of simultaneously performing NIR fluorescent imaging and PTA, were designed by conjugation of rod-shaped gold nanoparticles and Cy 5.5 NIR fluorescent molecules. The synthesized dual-modal GNRs were not only capable of NIR fluorescent imaging but also exhibited effective photothermal effects upon irradiation using an endoscopic NIR wavelength laser. A translational transgenic mouse model successfully demonstrated that dual-modal GNRs could target inflammatory polyps. Finally, NIRF endoscopic image-guided PTA with the dual-modal GNRs achieved precise ablation of polyps. This NIRF endoscopic image guidance approach combined with tumor-targeted fluorescent GNRs have the potential for the detection and treatment of CRC and various inflammatory diseases. Various new imaging techniques such as MRI, CT, photoacoustic imaging, and multifunctional nano-agents capable of highly efficient photothermal heating and imaging will further develop this approach for the treatment of CRC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Characterization of dual-modal GNRs. (a) UV-vis spectra of GNRs synthesized under different synthesis conditions (Inset: TEM images of synthesized GNRs). (b) TEM image of the PEG-coated GNRs. (c) Schematic illustration of dual-modal GNRs. After ligand exchange of the GNRs with PEG polymers bifunctionalized with thiol and amine groups, the NIR fluorescent probes are conjugated to the amine group at the end of the PEG. (d) Cy5.5 fluorescent image of the dual-modal GNRs. (e) Experimental setup for measurement of the photothermal effect by depth in artificial tissue (i.e., agarose gel). (f) Infrared thermal camera images of an agarose gel-containing dual-modal GNRs at 60 s after irradiation. (g) Temperatures recorded using infrared thermal camera images of the agarose gel at increasing distances from the laser irradiation site. (1–3) were measured using IR thermal camera images (Figure 1f) at a distance of 1, 1.5, and 2.0 cm from the laser source, respectively.

Figure 2.

NIR fluorescent endoscopic colon polyp detection with dual-modal GNRs. (a) Image of a fiber-type near-infrared laser (808 nm) device connected to the endoscope. (b) In vivo NIRF endoscopic imaging of tumor tissue (i.e., colon polyp). (Left) The colon polyps were imaged using a ProSense 750 fluorescent probe that specifically detected cathepsin enzyme activity in cancer cells. (Right) The accumulated dual-modal GNRs labeled with Cy5.5 in the same colon polyps were imaged through the NIRF endoscope. (c) Fluorescent immunohistology images showing the distribution of dual-modal GNRs and macrophages in the colon polyps. Cy5.5 labeled with GNRs (green) and fluorescent F4/80 antibody (red), a marker for macrophages, are imaged with fluorescent microscopy, respectively (63× magnification). (d) In situ IR thermal camera image of a small intestine with colon polyps from a transgenic mouse colon cancer model. (Left) Digital photograph of a mouse intestine with colon polyps. (Right) IR thermal camera image when NIR laser is applied to the dual-modal GNRs upon targeted delivery to a colon polyp.

Figure 3.

In vivo real-time endoscopic photothermal ablation of a colon polyp using targeted dual-modal GNR in a *TS4 CRE/APC^{lox 468}* colon cancer mouse model. Step 1. Real-time detection of accumulated GNRs (Cy5.5) in the colon polyps in the NIRF endoscope. Step 2. Real-time photothermal ablation of the NIRF endoscope-detected polyp with GNRs.

Figure 4.

Histological analysis of tissues treated with NIRF endoscopic image-guided PTA with dual-modal GNRs in the transgenic colorectal cancer mouse model. Representative (a) H&E- and (b) TUNEL-stained tissue sections of the treated mice. (c,d) Intravital microscopy of small polyps after iv injection of dual-modal GNRs (at 24 h after injection) and NIRF endoscopic image-guided PTA. Blue (DAPI: extracellular matrix), red (Cy5.5: dual-modal GNRs), and green (F4/80: macrophages or Gr1: neutrophils). Yellow arrows are indicating the interface area between a polyp and normal tissues.

Scheme 1.

Schematic Illustrations of NIRF Endoscopic Image-Guided PTA Therapy with Dual-Modal GNRs for the Treatment of Colorectal Cancer^a

^aIV injected GNRs are recognized as foreign by macrophages and internalized into macrophages through phagocytosis. The macrophages, including GNRs, are accumulated in tumor sites. The GNRs are imaged through an NIRF endoscope to specifically irradiate only the cancer cells with an NIR laser (i.e., 808 nm). The GNRs accumulated in the tumor tissue generate heat to ablate cancer cells upon endoscopic laser irradiation.