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Hyperthermia

Convertible Organic Nanoparticles for Near-Infrared Photothermal Ablation of Cancer Cells**

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Well-designed photothermal nanomaterials have attracted the interest of many scientists pursuing a better means to accurately diagnose cancer and assess the efficacy of treatment, because these materials enable therapies in which the tumor region is pin-pointed with a laser-guided light source without surgical intervention.[1–5] Two major subgroups of photothermal agents are currently available: gold-based nanostructures (nanoshells, nanocages, and nanorods) capable of inducing surface plasmon resonance (SPR), and carbon nanotubes that enable the photothermal ablation of cancer cells with near-infrared (NIR) light but do not damage normal human tissues.[2,6–9] The functional enhancement of these nanoparticles to incorporate payloads, such as chemotherapeutic drugs and/or biological substances, for tumor targeting, molecular imaging, and the destruction of cancer cells has been attempted.[1] Although considerable effort has been devoted to the fabrication of sophisticated nanostructures of this type, further optimization is required for the development nanoparticles with cytotoxic properties, such as the induction of oxidative stress.[8,10,11]

Herein, we demonstrate the feasibility of a novel organic photothermal agent based on polyaniline for the induction of hyperthermia in epithelial cancer. Polyaniline is biocompatible and has been used as an electroactive material for studying cellular proliferation.[12,13] A key advantage of polyaniline is that dopants (i.e., strong acids, Lewis acids, transition metals, alkali ions) for protonation generate an interband gap state between valence and conduction bands that induces the movement of electrons and decreases the excitation-energy level.[14–17] Thus, the optical-absorbance peak of polyaniline is red-shifted toward the NIR region as a result of its transition from the emeralidine base (EB) to the emeralidine salt (ES) during the doping process. The absorption of NIR light by polyaniline generates a substantial amount of heat energy that can be used for cancer-cell ablation (Figure 1).

Polyaniline was synthesized by using anilinium salts protonated by hydrochloride (HCl) and ammonium persulfate as an oxidant.[12,14] The chemical oxidative polymerization process was carried out for 6 hours at 4 °C and resulted in a dark-green precipitate (ES), which was purified by washing with copious amounts of deionized water. The synthesized ES was dedoped with sodium hydroxide to increase the solubility of polyaniline in the organic phase (chloroform) and to obtain a homogeneous nanoparticle size. Upon dedoping, the color of the synthesized polymer powder changed to purple (EB). The molecular weight of synthesized polyaniline EB was 5200 Da, as measured by gel permeation chromatograpy (polydispersity index: 1.1; see Figure S1 in the Supporting Information). To provide the required water solubility, we capped the hydrophobic EB polymers with PEGylated fatty acid (poly(ethylene glycol) stearate) by the nanoemulsion method (see the Supporting Information).[3,18] The polyaniline nanoparticles in the EB state (EB PANPs) were highly water-soluble owing to the outer polyoxyethylene chains (Figure 2a). Scanning electron microscopy (SEM) showed that EB PANPs have a smooth surface and a spherical shape (Figure 2b) and are monodisperse with a size of 115.6 ± 16.3 nm at the feeding amount of 10 mg of polyaniline (Figure 2c). As the feeding amount increased, the size of the PANPs increased, from (307.8 ± 32.4) nm at 50 mg of polyaniline to (412.7 ± 36.2) nm at 100 mg of polyaniline (see Figure S2 in the Supporting Information). However, these nanoparticles were relatively large and therefore too unstable in the aqueous phase for photothermal application.

To investigate the sensitivity of PANPs to acidic and oxidative species, we evaluated their optical properties and colloidal stability at varying pH values. In the absorption spectra of EB PANPs in phosphate-buffered saline (pH 7.4), a π–π^* transition of the benzene rings was observed at 435 nm, and charge transfer between quinoid and benzenoid rings was

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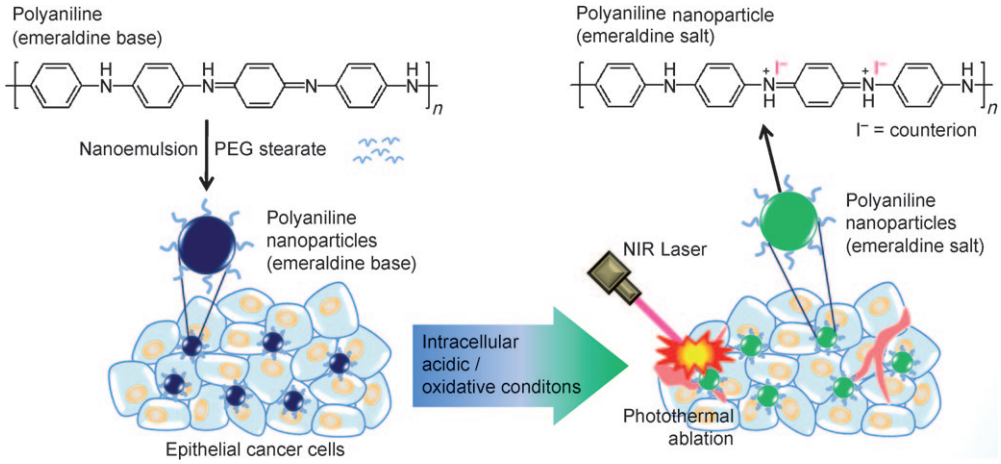

Figure 1. Schematic illustration of the preparation of organic photothermal agents based on polyaniline nanoparticles and their application for the photothermal ablation of epithelial cancer cells by NIR laser irradiation.

observed at 580 nm (Figure 2d; see also Figure S3 in the Supporting Information).[12] When EB PANPs were doped with phosphate-buffered saline at pH 1 (transition to ES PANPs), the color of the PANPs changed from dark purple to green (ES PANPs) without aggregation or precipitation (inset in Figure 2d). Moreover, the main absorption peak for charge transfer between quinoid and benzenoid rings was red-shifted as a result of increased electron-delivery efficiency. The π–π* transition of ES PANPs was analogous to that of EB PANPs (430 nm), but charge transfer between quinoid and benzenoid rings was observed only in the NIR region (810 nm). With this decrease in the pH value (to pH 1), the absorption of ES PANPs at 810 nm increased by approximately 187% from that observed for EB PANPs [at pH 7.4; $((ABS_{ES\,PANPs\,(pH\,1)} - ABS_{EB\,PANPs\,(pH\,7.4)})/ABS_{EB\,PANPs\,(pH\,7.4)}) \times 100$]. Consequently, ES PANPs are well-suited as a photothermal agent for use with NIR laser irradiation at 810 nm, which does not damage blood or normal tissue. However, the extremely low pH conditions (< pH 3) are difficult to generate in live cancer cells.[19] We studied the absorption spectra of PANPs after treatment with oxidative species (i.e., hydrogen peroxide and hydroxyl radicals), since there are many potential dopants in live cancer cells, such as protons, alkali ions, and oxidative species generated from mitochondria.[20] We verified the effectiveness of the doping process to form ES PANPs from EB PANPs by the treatment of EB PANPs with oxidative species as potential dopants (see Figure S4 in the Supporting Information). In the case of the hydroxyl radical, the absorption ratio ($\lambda_{810}/\lambda_{580}$) increased as the concentration of the oxidative species increased. However, there was no meaningful change with hydrogen peroxide. The degree of oxidative stress may be important. After all, the prepared PANPs might be doped by biological dopants (acidic environment and/or oxidative species from mitochondria) and exhibit strong NIR absorption. Thus, ES PANPs formed by convergence doping by biological dopants (intracellular protons and oxidative species) may ablate cancer cells by a photothermal effect.

The hydrodynamic size of ES PANPs [(121.3 ± 23.4) nm] after doping with phosphate-buffered saline (pH 1) did not significantly differ from that of EB PANPs (see Figure S5 in the Supporting Information), and the zeta potential of ES PANPs (at pH 1, (17.6 ± 3.4) mV) was similar to that of EB PANPs (at pH 7.4, (13.6 ± 3.7) mV). Also, we observed that ES PANPs maintained colloid stability for up to 15 days. After doping of the polyaniline core of PANPs, the outer polyoxyethylene shell still provided steric hinderance. Furthermore, the chemical structure of PANPs (both EB and ES states) was confirmed by their FTIR spectra: 1148 cm^{-1} (N=Q=N vibrations: stretching vibrations of quinoid rings), 1330 cm^{-1} (aromatic C–N stretching), 1467 cm^{-1} (C=C and C=N stretching of benzenoid rings), and 1563 cm^{-1} (C=C and C=N stretching of quinoid rings; see Figure S6 in the Supporting Information).

To investigate the hyperthermic potential of PANPs, we evaluated the amount of heat generated upon NIR laser irradiation (808 nm and 2.45 $W\,cm^{-2}$ for 5 minutes; Figure 3c). NIR irradiation of the solution of ES PANPs resulted in a greater temperature rise (54.8 °C) than that observed for pure water (6.6 °C). Thermal images recorded with an IR camera confirmed the hyperthermic effect (see Figure S7 in the Supporting Information): a distinct color change from blue (pure water) to deep red (solution of ES PANPs) was observed. Finally, the colloidal stability of ES PANPs was maintained after NIR irradiation.

We next evaluated the in vitro photothermal-ablation capacity of EB PANPs with A431 cells, an epithelial cancer cell line, to determine if the transition from EB PANPs to ES PANPs was induced by the biological dopants (i.e., protons and oxidative species) as protonation agents found in cancer cells.[21,22] First, the cell viability of A431 cells treated with PANPs at various concentrations was assessed. Significant inhibition of growth and proliferation was not observed up to a 10 $mg\,mL^{-1}$ concentration of PANPs (see Figure S8 in the Supporting Information). In the culture medium (the Dulbecco modified Eagle medium), EB PANPs exhibited a dark-purple color (Figure 3a), and a π–π* transition was observed in the absorption spectrum (Figure 3b). However, when EB PANPs were treated with A431 cells, they turned green (Figure 3a), and the main absorption peak was shifted to the NIR region (Figure 3b). A431 cells treated with EB PANPs exhibited a substantial red shift in the absorption spectrum (from 545 to > 700 nm) owing to a decrease in the band-gap energy as a result of the doping process (Figure 3b). In contrast, EB PANPs in a solution of fetal bovine serum (with an FBS concentration of up to 75%)

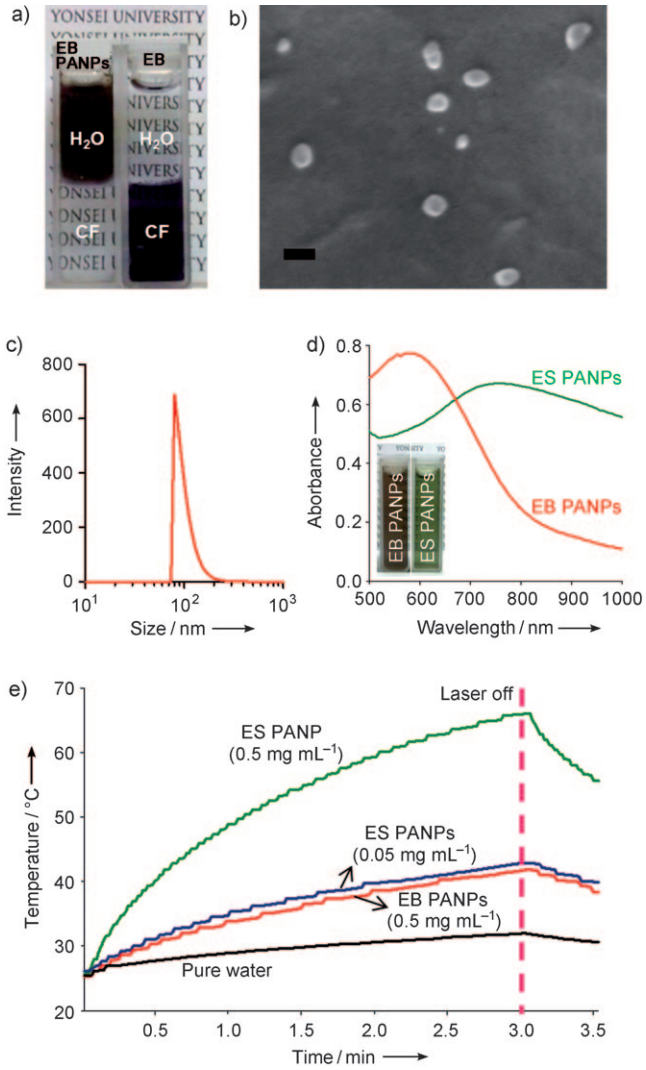

Figure 2. a) Solubility test of EB PANPs (left) and EB (right) in chloroform (lower phase) and water (upper phase). b) SEM image of EB PANPs (scale bar: 200 nm). c) Size distribution of EB PANPs. d) Absorption spectra and photographs (inset) showing the EB PANP and ES PANP states. e) Photothermal effect of the irradiation of pure water, EB PANPs, and ES PANPs with an NIR laser (808 nm, 2.45 W cm^{-2}). The NIR laser was turned off after 3 min.

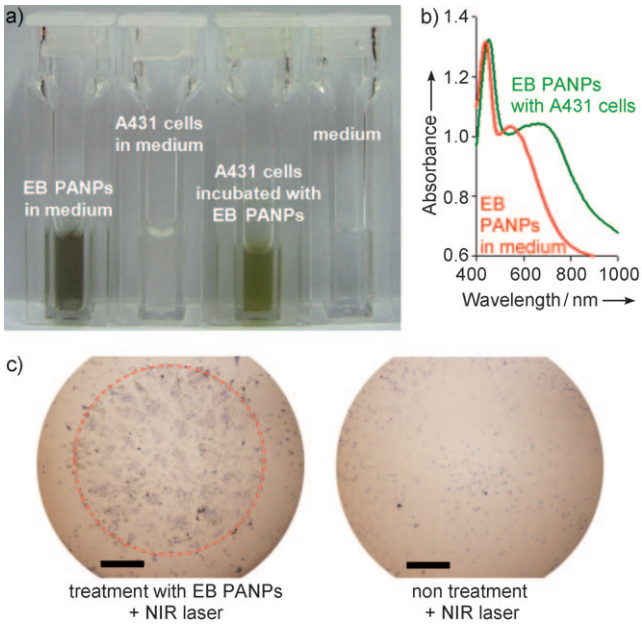

Figure 3. a) Photographs of EB PANPs in culture medium, A431 cells in culture medium, A431 cells incubated with EB PANPs (transition to ES PANPs), and the free medium. b) Absorption spectra of A431 cells treated with EB PANPs (transition to ES PANPs) and free EB PANPs in culture medium. c) Microscopic images of A431 cells treated with EB PANPs (left) and untreated control cells (right) after NIR laser irradiation (808 nm, 2.45 W cm^{-2}) for 5 min (scale bar: 200 μm).

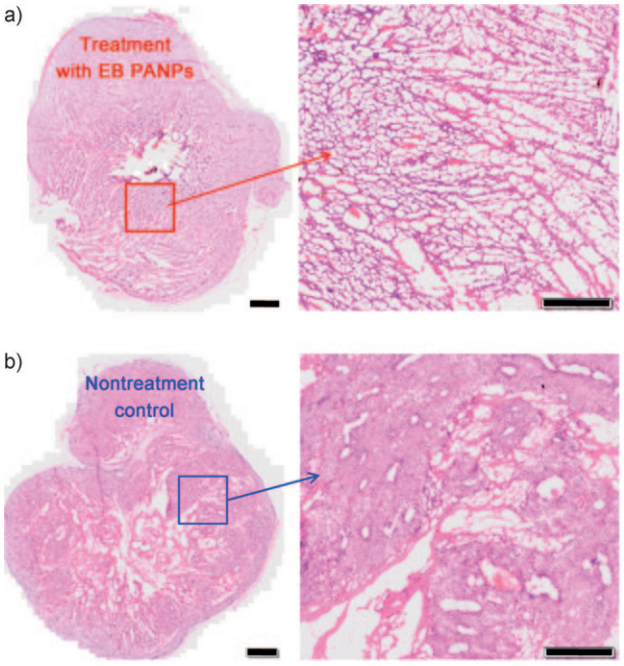

Figure 4. Histological examination of tumor-tissue sections: a) after treatment with EB PANPs and NIR irradiation; b) untreated control. Scale bars: 1 mm (lower magnification, left) and 500 μm (higher magnification, right).

did not cause a similar shift (see Figure S9 in the Supporting Information). Furthermore, we confirmed that EB PANPs cannot be transited to the ES state by treatment with A431 cell lysate (see Figure S10 in the Supporting Information). Together these results demonstrate that EB PANPs were efficiently doped by biological dopants (i.e., protons and oxidative species) in the intracellular environment of this cancer cell line. Next, we investigated the ability of EB PANPs to promote photothermal ablation of A431 cells by NIR laser irradiation ($\lambda = 808$ nm, 2.45 W cm^{-2}, for 5 min). EB PANPs mediated substantial cell destruction, as determined by staining with trypan blue (red circle in Figure 3c, left); however, A431 cells were not damaged by NIR irradiation in the absence of EB PANPs (Figure 3c, right).

Finally, we tested the photothermal ablation of A431 cells in vivo. A431 cells were transplanted into the proximal thigh region of nude mice, and EB PANPs (0.5 mg mL^{-1} in 200 μL) were injected into the tumor site and exposed to NIR laser irradiation ($\lambda = 808$ nm, 2.45 W cm^{-2}). Both in vivo and ex

vivo absorption images clearly showed strong absorption of the NIR light ($\lambda = 730$ nm) by the tumors injected with EB PANPs (see Figure S11 in the Supporting Information). Histological examination of tumors treated with EB PANPs (Figure 4a) showed severe cellular damage (pyknosis and karyolysis) as well as blood-vessel damage in comparison with an untreated control (Figure 4b). The photothermal energy generated by PANPs upon exposure to NIR light might induce the breakdown of cellular components that block DNA and/or RNA synthesis necessary for cellular growth and proliferation. Specifically, photothermal treatment degrades proteins and causes the depolymerization of cytoskeletal filaments; these processes can also lead to cell death. Together, our results demonstrate that the combination of PANPs and NIR light is a highly effective and feasible photothermal-ablation cancer therapy.

In summary, we have formulated organic PANPs as a novel photothermal agent for cancer-cell ablation. The water-soluble PANPs exhibited good colloidal stability and NIR absorption that depended on the pH value and the presence of oxidative species in an intracellular environment. Moreover, both in vitro and in vivo assays confirmed that EB PANPs were transformed into ES PANPs that absorbed NIR light by a biological doping process. NIR irradiation of tumors treated with PNAPs resulted in effective ablation of cancer cells. Thus, these nanoparticles appear promising for cancer therapy. Continued optimization of PANPs by the addition of molecules to target the particles to tumor tissues would add a further degree of specificity and enhance the safety of this therapy for use in humans.

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