In vivo photothermal treatment with real-time monitoring by optical fiber-needle array

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Abstract: Photothermal treatment (PTT) using gold nanoshells (gold-NSs) is accepted as a method for treating cancer. However, owing to restrictions in therapeutic depth and skin damage caused by excessive light exposure, its application has been limited to lesions close to the epidermis. Here, we demonstrate an in vivo PTT method that uses gold-NSs with a flexible optical fiber-needle array (OFNA), which is an array of multiple needles in which multimode optical fibers are inserted, one in each, for light delivery. The light for PTT was directly administrated to subcutaneous tissues through the OFNA, causing negligible thermal damage to the skin. Enhancement of light energy delivery assisted by the OFNA in a target area was confirmed by investigation using artificial tissues. The ability of OFNA to treat cancer without causing cutaneous thermal damage was also verified by hematoxylin and eosin (H&E) staining and optical coherence tomography in cancer models in mice. In addition, the OFNA allowed for observation of the target site through an imaging fiber bundle. By imaging the activation of the injected gold-NSs, we were able to obtain information on the PTT process in real-time.

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1. Introduction

Photothermal treatment (PTT) is a therapeutic method that destroys target tumors by converting photon energy into heat via nanoparticles as photosensitizing agents that are activated when exposed to light of a specific wavelength [1–5]. We previously suggested in vitro and in vivo studies confirming the feasibility of PTT using nanoshell-loaded macrophages as live cell vectors [5,6]. Peritumoral-injected macrophages acting as nanoparticle carriers effectively moved into a xenograft tumor target site in live mice. Moreover, this method was used to verify that the photothermal effect of a near-infrared (NIR) laser destroyed not only the macrophages themselves but also neighboring cancer cells while causing minimal damage to adjacent tissues.

To deliver the source of activation energy (light) to a tumor site with nanoparticles, it must sufficiently penetrate healthy tissues [7,8]. Unfortunately, the transport distance of light in biological tissues is limited by scattering and absorption [9]. Moreover, percutaneous light delivery will inevitably elicit undesirable damage to overlying tissues. Therefore, current phototherapies can only be used as low-level light therapy (LLLT) to treat lesions in shallow, superficial layers of the skin [10,11]. In order to overcome these problems, several groups have investigated treatment of tumors via hyperthermia using deep penetrating NIR lasers.
with wavelengths in a desirable optical window of tissues for deep penetration of light into tissues [12–14]. Moreover, optical fibers, optical microneedles [7,15], optical fibers with diffusers [16,17], and implantable light-guiding fiber materials [18] have been developed for ex vivo and in vivo applications. In principle, these approaches have the potential to treat embedded tumors, but clinical approaches are not yet feasible. Furthermore, several imaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI), X-ray, and optical coherence tomography (OCT), have been combined with PTT to improve the effectiveness of cancer treatment [19–21]. However, it has until now been impossible to observe the PTT process in real time at target tumor sites.

In this paper, we demonstrated a method of enhanced light delivery deep into tissues that is associated with negligible cutaneous thermal damage and improves the efficiency of in vivo PTT using gold-NSs. To achieve this aim, we fabricated an optical fiber-needle array (OFNA), in which multimode optical fibers were inserted into fine syringe needles to deliver light energy to the tumor site where the PTT is required. The capacity of the OFNA to transport light deep region with almost no loss was verified using an artificial tissue. After performing PTT by OFNA, we investigated the photothermal effect on the skin by swept source OCT (ss-OCT) to confirm that thermal damage was minimized. The destruction of tumor masses after PTT by OFNA was proven by immunohistochemistry. During the treatment, we also observed the PTT process in real-time at the target site through an additional port in a needle containing an image fiber bundle located at the center of the OFNA. By collecting the signal generated by the activation of the injected gold-NSs, the OFNA can provide data necessary to determine whether PTT is being performed appropriately.

2. Materials and methods

All animal experiments were performed according to a protocol approved by our Institutional Animal Care and Use Committee.

2.1 Preparation of gold nanoshells

The core silica particles for gold-NSs were synthesized following a modified Stöber method. In brief, an ammonium hydroxide solution (5.5 mL, 30% NH₃ for the NH₄OH assay) was added to 50 mL of ethanol with vigorous stirring, to which 1.5 mL of TEOS was added in a dropwise manner. The mixture was stirred continuously for 2 hours, then the formed silica particles were washed and dispersed in water. The surface of the silica particles (50 mL in aqueous solution) were amine-activated using APTMS (20 μL) for 6 hours. The tiny gold nanoparticles were separately synthesized by rapidly combining an aqueous HAuCl₄ solution (2 mL, 27 mM) with a mixture containing pure water (45 mL), aqueous NaOH solution (0.5 mL, 1 M), and THPC prepared by adding 12 μL of an 80% THPC aqueous solution to 1 mL of water. After 5 min, the amine-functionalized silica nanoparticle solution (100 μL) was gently added to 1 mL of this dark brown gold nanoparticle solution and the mixture was shaken for 1 hour. The silica-gold nanoparticle composites were dispersed in pure water, and 20 μL of this solution was combined with 4 mL of the growth solution (1.5 mL of 27 mM HAuCl₄ + 100 mL of 1.81 mM K₂CO₃) and 27 μL of formaldehyde to promote shell growth. For more information on the preparation of the gold-NS, refer to the refs [5,6].

2.2 Preparation of cancer cells and gold-NSs loaded peritoneal macrophages

An anaplastic thyroid carcinoma cell line (FRO) which is derived from a human pharyngeal cancer specimen was cultured in a mixture of 500 mL Roswell Park Memorial Institute (RPMI) 1640 (Gibco BRL, Grand Island, New York) and 50 mL fetal bovine serum (Gibco BRL) in an incubator (37°C, 5% CO₂). In addition, peritoneal macrophages were prepared from 6- to 8-week-old outbred mice from the Institute for Cancer Research (ICR) that had been intraperitoneally injected with 10 mL of phosphate-buffered saline solution (PBS). The
macrophages were isolated from the PBS cell suspension by adding a red blood cell (RBC) lysis buffer followed by centrifugation. These studies were performed according to an Animal Medical Center Institutional Review Board approved protocol.

In the previous study [5,6], we found that the optimal concentration of gold-NSs was around 27.5 pM and the optimal number of macrophage cells was $2 \times 10^4$ for PTT performance. The gold-NSs suspension was co-incubated with peritoneal macrophages with gentle shaking for 1 hour. Finally, we obtained a cell suspension with $1 \times 10^4$ cells/mL of gold-NS-loaded macrophages.

### 2.3 Xenotransplantation of the cancer cell line

The FRO cancer cell line was diluted to $10^8$ cells/mL, and the cells were injected subcutaneously into the flanks of nude mice (BALB/c-nu/nu, male, aged 5 weeks) using a syringe. The animals’ body weights and physical symptoms were monitored during the experiments. Tumor size was measured three times per week, and PTT was performed when the maximal diameter of the tumor became greater than 10 mm.

### 2.4 Design of the optical fiber-needle array (OFNA)

The OFNA consists of eight multimode optical fibers and a single imaging fiber bundle. The multimode fibers (F-MFC, New Port) deliver NIR light to a predetermined target depth within a lesion for PTT. The diameter of the individual fiber is 650 μm (core diameter: 550 μm) and its length is about 30 cm. The imaging fiber bundle (FIGH-06-300S, 0.39 N.A., Fujikura) has 6000 fibers with cores 5 μm in size. The total diameter of the fiber bundle is 400 μm (image circle diameter: 270 μm). On the output side of the OFNA (facing a target), eight multimode fibers were inserted into eight syringe needles (19 gauge) for easy penetration through tissues. The length of the needle used is about 4 mm, which is sufficient to reach target sites in tissues. We also made a holder for the fiber-needle array that consisted of a circular plate 10 mm in diameter and 1 mm thick that had nine holes arranged in a 3 × 3 square form, as shown in the inset in Fig. 1(b). The optical fiber-needle array is located in the eighth of the nine holes along the edge of the square. The imaging fiber bundle was inserted into a 21-gauge needle and located and mounted in the center hole. On the input side, the eight multimode fibers are used for light delivery and the imaging fiber bundle is used for collecting the light scattered from the target site. Since the OFNA is equipped with the imaging fiber bundle, an image of an object in the plane adjacent to the distal surface of the fiber bundle can be obtained. For this purpose, an imaging system configured with an objective lens (10x, 0.25 NA), a tube lens, and a camera (FL3-U3 13Y3M, Point Grey) was placed as shown in Fig. 1(a).

### 2.5 Swept-source optical coherence tomography

We used an ss-OCT system with a fiber-based Mach-Zehnder interferometer. A commercial swept laser (Santec, Inc., Japan) with a full width at half maximum (FWHM, $\Delta \lambda$) of 100 nm centered at 1064 nm that operates at a 100 kHz sweeping rate. The axial resolution was about 7 μm in air. A dual-balanced photodetector (Thorlabs Inc., USA) was used to remove DC and auto-correlation noise, and also to enhance the interference signal at the detector. A 12-bit waveform digitizer (ATS9360; Alazar Technologies, Inc., USA) with a sampling rate of 1.5 GS/s was used to acquire the signal. The usual optical power in the sample arm was 2.0 mW. A laboratory-built program developed in the C language was used to produce the final OCT images.

### 2.6 Photothermal treatment of xenografts in nude mice

We conducted an in vivo PTT experiment as shown in the illustration in Fig. 1(b). The NS-loaded macrophages were applied to a xenografted nude mouse using a peritumoral injection method, in which 10 μL of NS-loaded macrophages ($1 \times 10^7$ cells/mL) was injected into four
different locations around the xenograft tumor mass. An NIR laser with a center wavelength of 960 nm was used as a thermal light source. The laser output generated a loose focus through a collection lens at the focal plane where all the proximal ends of the multimode fibers were placed. The output intensity at the distal end of each fiber was measured as 0.5 W/cm². After inserting the OFNA about 3 mm deep into the tumor mass, the laser was irradiated through the fibers for 1 minute. During the laser irradiation, the progress of the treatment at the target site was observed via the imaging fiber bundle. After PTT, the xenograft tumor mass was resected and examined by conventional histologic method using hematoxylin and eosin (H&E) staining.

3. Results

3.1 Enhancement of light delivery by the OFNA

We first investigated the efficiency of light delivery through the OFNA by measuring the intensity profiles of the light within a tissue phantom, which was made of scattering particles embedded in polydimethylsiloxane (PDMS) as a host medium. Zinc-oxide (ZnO) nanoparticles were evenly dispersed in PDMS and cured in a cube-shaped container with a volume of about 23³ mm³. The scattering mean free path was measured to be about 110 μm. For this test experiment, we fabricated another OFNA with a simpler configuration consisting of three optical fiber-needles at 5 mm intervals. After inserting the simple OFNA into the PDMS phantom, the NIR laser beam was illuminated by the simple OFNA. The light exiting from the end of the tips of the OFNA needles spread diffusely into the PDMS. Since the location where the needle tips were inserted was 1 mm away from one edge of the PDMS phantom, the internal distribution of the light intensity could be easily observed from the side. An imaging setup was placed on the side of the PDMS phantom, and then the light distribution was measured at different insertion depths of 0, 5, and 10 mm from the surface.

First, in the experiment with a depth of 0 mm, the NIR laser was sent through the simple OFNA to the PDMS phantom without inserting the optical fiber-needles. The output intensity of the laser was adjusted to 1 W/cm² at the distal ends of the fibers. The light distribution observed from the side of the PDMS phantom is presented in Fig. 2(a). The incident light on the surface diffused through the phantom and the intensity eventually decreased in the depth direction. Figure 2(d) shows the profile of the light distribution along the depth direction along the red dotted line in Fig. 2(a). The position of maximum intensity was observed to be located at about 3 mm below the surface, not on the surface, owing to the 1 mm mismatch of
the optical axis and the plane for the observation. Figures 2(b) and 2(c) show the light distributions measured when the insertion depth of the simple OFNA was 5 mm and 10 mm, respectively. The intensity of the irradiation was kept at 1 W/cm² at the needle tips. As shown in Fig. 2(d), maximum light intensity was seen near the insertion depth of the OFNA. Note that in all three cases, the maximum intensity was maintained at similar levels. This means that the OFNA can deliver almost all of its light energy to the target region, irrespective of its depth. Figure 2(e) shows the lateral profiles of the light distributions for the three cases at a depth of 10 mm from the surface. The intensity profile of the OFNA with a 10 mm insertion depth exhibited the maximum distribution. This verifies that the light delivered by the OFNA is most effective when the needles reach a specific target depth (10 mm insertion depth in this particular case).

Next, we investigated the capacity of the OFNA to perform real-time monitoring of the activation of injected gold-NSs in tissues. For this purpose, the activation signal generated by the gold-NSs embedded in a PDMS phantom was captured by the imaging fiber bundle of the OFNA while the NIR laser was irradiated through the multimode fibers of the OFNA. As a control, 10% of crosslinking agent without gold-NSs was added to the PDMS phantom in a liquid state and mixed well. The whole liquid was baked in an Eppendorf tube (1.5 ml) at 60°C for 4 hours to achieve solidification. For a comparison experiment, a tissue phantom embedded with gold-NSs was also prepared. Gold-NSs (0.3 ml, 27.5 pM) and 1 ml of crosslinking agent were mixed well in another PDMS phantom and the whole liquid was baked under the same conditions. For the purpose of a simple demonstration, an optical fiber-needle array with two channels, one for a multimode fiber for NIR laser delivery and the other for an imaging fiber bundle to allow observation of the gold-NSs, was inserted into the fabricated PDMS phantoms, as illustrated in Fig. 3(a). After irradiation of the NIR laser with light at an intensity of 1 W/cm² at the distal tip, the light captured by the imaging fiber bundle was recorded by a camera. Figure 3(b) shows the image taken after NIR laser irradiation in the tissue phantom without the gold-NSs. Because of multiple scattering introduced by the nanoparticles embedded in the phantom, a signal that was almost uniformly distributed over...
the fiber bundle was observed. By contrast, as presented in Fig. 3(c), the image taken with the tissue phantom with gold-NSs shows distinct differences, with a strong spotty distribution, especially in the upper region, even with the same amount of laser illumination. This is evidence for the activation of the gold-NSs by NIR laser irradiation. Visualization 1 demonstrates the real-time monitoring of the activation of the gold-NSs.

Fig. 3. Real-time monitoring of activated gold-NSs by the OFNA. (a) A schematic of the test experiment with the simplified OFNA. The image in the right side is a zoomed in view of the region marked with a red dotted circle in (a). The red arrow denotes the NIR laser illumination from the tip of the needle and the white arrow represents light scattered by the activation of the gold-NSs. (b) An image taken in a tissue phantom by the simplified OFNA without gold-NSs injection. (c) The same as in (b), but with gold-NSs. The glittery spots in the upper region are caused by the light generated by the activation of the gold-NSs (Visualization 1). Scale bar, 100 μm.

3.2 Evaluation of photothermal damage on skin by swept-source OCT

We investigated the photothermal effect of laser irradiation on skin during PTT with and without the OFNA. To examine the extent and degree of the photothermal damage, we observed the structure of the skin for each case using an ss-OCT system. Figure 4(a) is a representative OCT image of normal skin tissue, which clearly shows the layers of the epidermis and dermis stratum. Figure 4(b) represents an image of a tumor site. Unlike the normal case, the layered structure is unclear because the implanted tumor mass occupies a certain volume in the dermis of the subcutaneous tissue, producing pressure toward the skin’s surface.

We first performed PTT on a tissue with a tumor using the OFNA. We inserted the needles about 3 mm deep into the tumor site, and the NIR laser was irradiated for 2 minutes through the OFNA with an output intensity of 1 W/cm² at the needle tips. After completion of the PTT, the OFNA was removed and the treated site was imaged with the ss-OCT to investigate the effect of PTT on the skin. As shown in Fig. 4(c), a hole created by needle insertion was found near the surface of the tissue. Though there is physical damage done by the needle, its range is limited only to the region where the needle was inserted. Two weeks later, we imaged the same area of tissue again to assess the status of the tissue after the wound had healed. As presented in Fig. 4(d), the physical damage done by the needle had completely healed and no more needle marks were found. Furthermore, the anatomical structure of the skin tissue appears to be quite similar to that of normal tissue after recovery.
Next, for PTT without the OFNA, the NIR laser was directly illuminated on the skin of the tumor site with an intensity of 1 W/cm² for 2 minutes. After conventional PTT, we observed that the epidermis stratum had significantly shrunk in the OCT image, as shown in Fig. 4(e). Moreover, the dermis stratum had become an empty space between the layers, as indicated by the red dotted box in Fig. 4(e), which seemed to have been swollen by the thermal damage caused by direct NIR laser irradiation. Two weeks after PTT, indurated tissue had developed on the skin with a crust, as presented in Fig. 4(f). Wound healing seems to be slower than that following OFNA treatment. Extensive and massive cellular destruction was observed within the tumor site as a result of performing PTT; however, a serious skin burn occurred mainly around the illumination site that remained after recovery.

3.3 Effect of photothermal treatment with the OFNA

Before we assessed the effect of PTT via OFNA, we performed histochemical investigation of normal skin tissue using conventional hematoxylin and eosin (H&E) staining. Figure 5(a) shows an H&E-stained image of normal skin tissue, which is the same tissue as shown in Fig. 4(a), revealing the characteristic features of normal dermis stratum. In order to evaluate the effect of PTT with the OFNA, we prepared xenografted nude mice with tumor masses. We injected gold-NSs into the peritumoral sites and waited for approximately 48 hours for it to spread through the tumor masses [6]. To perform PTT, we stuck the OFNA on the tumor site through the skin and then illuminated the NIR laser through the fibers for 1 minute at an intensity of 0.5 W/cm² per fiber (see Visualization 2). The total amount of light energy delivered to the tissue during PTT was 0.38 J. The irradiation intensity of the NIR laser and the treatment duration were set at half the respective values used in our previous study with direct laser administration [6]. Immediately after PTT, we removed the OFNA from the tumor site and found that there was no swelling and no burn on the skin (see Visualization 2). Two weeks after the treatments with and without the OFNA, the damage at the tumor sites had almost healed. In order to examine the effect of PPT, the tumor masses were resected and H&E stained for histochemical inspection. In both cases, the cancerous cells had all been destroyed by the treatments, as shown in Fig. 5(b) and (d) (with and without the OFNA, in that order). When the OFNA was used, no needle holes were found on the surface of the tissue. Beneath the skin, normal fibrotic tissue without tumor cells was observed, as shown in
the zoomed-in view in Fig. 5(c). It shows many lymphocytes with small size and scarce cytoplasm instead of tumor cells.

For the case of the regular PTT, the intensity of the NIR laser used to irradiate the tumor site was 1 W/cm² and the duration of irradiation was 2 minutes; thus, the total amount of light energy that reached the skin was 227 J. These parameters, which equated to 600 times more energy than that required when the OFNA was used, were verified as the optimum values for PTT by direct laser irradiation in our previous study [6]. Due to the restrictions on light delivery caused by multiple scattering in tissues, excessive laser intensity was required for administration of light to the target site through the surface of the skin. From the expectation based on the tissue optical properties, several hundreds of laser power would be required for this direct irradiation to deliver the same amount of light energy with that by OFNA to the target depth 3 mm deep in the tissues. Immediately after the regular PTT, severe swelling and a skin burn were observed on the tumor site, as reported in Ref [6]. Two weeks later, the tumor mass was resected and H&E stained. The damages to the PTT site had almost recovered and there were no tumor cells, as shown in Fig. 5(d) and the zoomed-in view in Fig. 5(e). However, we observed a ballooning area of degeneration, indicated by yellow arrows, and a disordered structure in the dermis stratum of the skin layer (Fig. 5(d)). Although extensive and massive cellular destruction was observed in the tumor, the skin developed into indurated tissue with a crust and then eventually obtained permanent scars from being burned.

When we compared the extent and degree of thermal damage in both cases, using the OFNA for PTT results in not only the absence of a superficial burn but also less deformation in the internal structure of the target tissue. This confirms that the OFNA has a therapeutic effect on the desired lesion and is associated with minimal damage.

3.4 Real-time monitoring of the activation of gold-NSs

Usually, PTT is performed in a blinded situation where no information on treatment beneath the skin can be obtained. We also demonstrated real-time monitoring of the PTT using a fiber bundle placed at the center of the OFNA. During PTT with the OFNA, we could visualize around the target region and observe the activation of the gold-NSs. Figure 6(a) shows a
picture taken while performing PTT with the OFNA in a nude mouse. First, we observed the signal returning through the fiber bundle while performing PTT with the OFNA in a normal mouse without gold-NSs injection. The PTT parameters were the same as those in Sec. 3.3. As shown in Fig. 6(b), we observed a low level of light scattering, which occurred only near the tissue. By contrast, when we performed PTT on a tumor site with gold-NSs injection, the light scattering became much stronger owing to the activation of the gold-NSs, as shown Fig. 6(c). Consequently, real-time monitoring of the target lesion through the OFNA can provide visual information on the progression of the performed PTT.

![Fig. 6. Real-time monitoring of PTT via the OFNA. (a) In vivo PTT performed with the OFNA at a tumor site in a mouse. (b) An image taken by the fiber bundle of the OFNA while PTT was performed on a nude mouse without gold-NSs injection. (c) The same as in (b), but with gold-NSs injection at the tumor site. A strong signal caused by the activation of the gold-NSs is evidence for the appropriate progression of PTT. Scale bars, 10 mm and 100 μm for (a) and (b), respectively. Visualization 2](image)

4. Summary and conclusion

We developed the OFNA as a tool for efficient delivery of light energy through a tissue for in vivo PTT assisted by gold-NSs. An NIR laser was transported through multimode optical fibers inserted into a syringe needle array. By sticking the fiber-needle array through a tissue, light energy could be directly administrated to the subcutaneous target lesion. Since the light did not undergo multiple scattering and absorption during delivery through the optical fibers, the light was able to reach the region where the treatment was required with almost no energy loss. We verified the capacity of OFNA for enhanced light delivery by investigation of the internal light distribution within a tissue phantom. We also confirmed that PTT treatment using the OFNA resulted in the destruction of cancerous cells in gold-NSs-injected tumor masses in mice. Compared to the direct laser irradiation method used in our previous study, using the OFNA for PTT required less laser power and a shorter treatment time. Owing to the superior light transport efficiency, the OFNA produced similar treatment results with only 0.17% of the light energy necessary for the usual method.

Furthermore, use of the OFNA minimized the thermal damage caused by laser irradiation of skin tissue. Because fine needles were used to administer light to the target lesion, the light could reach deep into tissues without interacting with the skin. Using sweep-source optical coherence tomographic method, we confirmed that no thermal damage had been done to the skin immediately after the PTT and all scars from fine needle puncture disappeared after two weeks of recovery. This is a distinct characteristic of PTT using the OFNA compared with PTT performed in the usual way: the negative consequences are vastly reduced.

In addition to its functional features for PTT, the OFNA allows for real-time observation of the PTT process at the target site through the fiber bundle placed at its center. By capturing the signal generated by the activation of the injected gold-NSs, the OFNA allowed real-time visual guidance that aided in determining whether PTT was being performed properly.
The OFNA may potentially improve the performance of existing PTT. Lossless light delivery will reduce the dose of light required for performing PTT, and direct energy administration will minimize thermal damage to the skin. These features will make it possible to perform efficient and repetitive PTT. In addition, the visual probe can provide additional information on the internal process of PTT as it is being performed. Although in its current state, the OFNA offers only indirect guidance, we envision adding high-resolution imaging capability based on wavefront control [22–24], thereby ensuring visibility of the target site. This will facilitate in vivo on-site diagnosis and immediate PTT for target diseases, such as primary tumor sites, regional metastatic lymph nodes, and cancerous lesions in deep tissues.

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