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Propagation characteristics of laser-induced stress wave in deep tissue for gene transfer

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ABSTRACT

Propagation characteristics of laser-induced stress waves (LISWs) in tissue and their correlation with properties of gene transfection were investigated for targeted deep-tissue gene therapy. LISWs were generated by irradiating a laser-absorbing material with 532-nm Q-switched Nd:YAG laser pulses; a transparent plastic sheet was attached on the absorbing material for plasma confinement. Temporal pressure profiles of LISWs that were propagated through different thickness tissues were measured with a needle-type hydrophone and propagation of LISWs in water was visualized by shadowgraph technique. The measurements showed that at a laser fluence of 1.2 J/cm^2 with a laser spot diameter of 3 mm, flat wavefront was maintained for up to 5 mm in depth and peak pressure P decreased with increasing tissue thickness d; P was proportional to $d^{-0.54}$. Rat dorsal skin was injected with plasmid DNA coding for reporter gene, on which different numbers of excised skin(s) was/were placed, and LISWs were applied from the top of the skins. Efficient gene expression was observed in the skin under the 3 mm thick stacked skins, suggesting that deep-located tissue such as muscle can be transfected by transcutaneous application of LISWs.

1. Introduction

Gene therapy has been regarded as a promising treatment for many human diseases, such as cancer and neuromuscular disorders [1,2]. Viral vectors have been widely used so far because of their high transduction efficiency, but their clinical application is limited due to poor spatial controllability of transduction [3], serious immune responses [4] and the difficulty in producing large amounts of pure virus. Gene therapy for many diseases including neurodegenerative diseases and spinal cord injury requires targeted gene transfer, for which various physical gene transfer methods, such as electroporation and microbubble ultrasound, have been investigated. However, further improvement in transfection efficiency and targeting characteristics is required.

Laser-based gene transfection is attractive as a new method for targeted gene therapy because of the high spatial controllability of laser energy. In addition, since laser can be delivered through an optical fiber, catheter-mediated gene delivery system may come into practical use. However, treatment of deep tissue is difficult when direct laser irradiation is used due to the limited penetration depth of laser light in tissue. We have been investigating gene transfection by the use of laser-induced stress waves (LISWs). Since LISWs can be efficiently propagated in tissue, deep-located tissue can be treated with this method. We previously demonstrated that reporter genes can be transferred to various types of cells *in vitro* and rat skin and mouse brain *in vivo* by the use of LISWs that were generated by irradiating a solid target with 532-nm Q-switched nanosecond Nd:YAG laser pulses [5–9]. Currently, we aim at efficient and targeted gene delivery to deep tissue, such as muscle and spinal cord, for which propagation characteristics of LISW in tissue should be revealed. In this study, propagation of LISWs was visualized in water as a tissue phantom by shadowgraph technique. In addition, we measured pressure characteristics of LISWs as a function of tissue depth by using excised rat skins. Correlation between the propagation characteristics of LISW and transfection efficiency was investigated.

2. Experimental

Laser-induced stress waves (LISWs) were generated by irradiating a target, an 8.0 mm diameter, 0.5 mm thick black natural rubber disk, with a 532-nm Q-switched Nd:YAG laser (Brilliant b, Quintal; pulse width, 6 ns FWHM). Laser pulse was focused with a plano-convex lens to a 3 mm diameter spot on the target; a 1.0 mm thick transparent polyethylene terephthalate sheet was bonded on the top of the target to confine laser-induced plasma for increasing LISW impulse [10].

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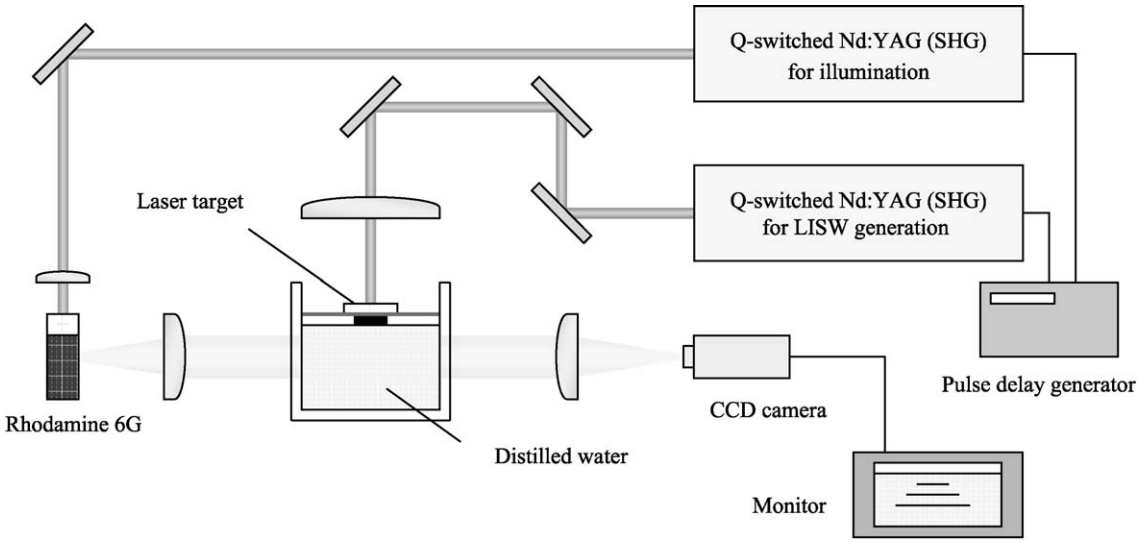

Fig. 1. The experimental setup for shadowgraphing the propagation of LISWs.

Fig. 1 shows a schematic diagram for shadowgraphing the propagation of LISWs. A transparent plastic container having a volume of 100 mm × 100 mm × 100 mm was filled with distilled water at 25 °C; on the water surface, a laser target was placed and the target was irradiated with laser pulses from the Q-switched Nd:YAG laser described above. Shadowgraph photographs of a LISW propagated through the water were taken by a CCD camera (XC-7500, Sony) with a zoom microscope lens as a function of time, for which fluorescence from a dye solution (Rhodamine-6G in ethanol) excited by an another 532-nm Q-switched Nd:YAG laser (Surelite I-10, Hoya Continuum; pulse width, 6 ns FWHM) was employed as back illumination. Imaging time point was controlled by changing a time interval between laser irradiation for LISW generation and photograph exposure; the interval was controlled by adjusting a trigger relay via a digital delay pulse generator (DG535, Tokyo Instruments). The image sequences were obtained, based on the reproducibility of the events by repeating the experiment under the same conditions.

Fig. 2(a) shows the experimental setup for the measurement of temporal pressure profiles of LISWs, in which a PZT needle-type hydrophone with a 1.0 mm diameter sensitive area (HNR-1000, Onda) was used. Output signals from the hydrophone were recorded by a digital oscilloscope (TDS4874D, Tektronics). A small portion of output pulses from the Q-switched Nd:YAG laser for LISW generation, which was detected with a biplanar phototube (R1328U-52, Hamamatsu Photonics), was used to trigger the oscilloscope to measure pressure temporal profiles of LISWs. Temporal profiles of LISWs were measured under the different thickness excised skin tissues, as shown in Fig. 2(b). The thickness was changed by stacking different number of skins which were excised from the back of male Sprague–Dawley rats weighting 300–380 g (Japan SLC), for which they were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg animal weight) and their dorsal hairs were clipped and depilated. The size of the excised skins was 15 mm × 15 mm × ~1.0 mm in thickness and their subcutaneous fat was removed before used for experiment. Ultrasound conductive gel was used to match acoustic impedances of a laser target (rubber), a protection film of the hydrophone and skins.

In order to examine the capability of LISWs for deep tissue transfection, we evaluated gene expression level in skin that was placed under the stacked excised skin(s) *in vivo*. Male Sprague–Dawley rats weighting 300–380 g (Japan SLC) were anesthetized and their dorsal hairs were clipped and depilated in the same manner as the pressure measurement experiment; afterward plasmid DNA encoding firefly luciferase (20 μl, 0.1 μg/μl) was intradermally injected with a 27-G needle syringe. The plasmid DNA driven by the cytomegalovirus (CMV) promoter was provided by Dr. Kaneda [11]. The plasmid (DNA) was transformed into competent *Escherichia coli* cells using standard procedures and then amplified and purified on a Qiagen column. The concentration of the vector in a TE solution (10 mM Tris and 1 mM EDTA, pH adjusted to 8.0) was 0.1 μg/μl. Excised skin(s) was/were placed on the plasmid injection site and a laser target was placed on the top surface of excised skin(s), as shown in Fig. 3. Ultrasound conductive gel was used between the target bottom surface and the top excised skin surface and between stacked skins to ensure acoustic impedance matching. The target was irradiated with a

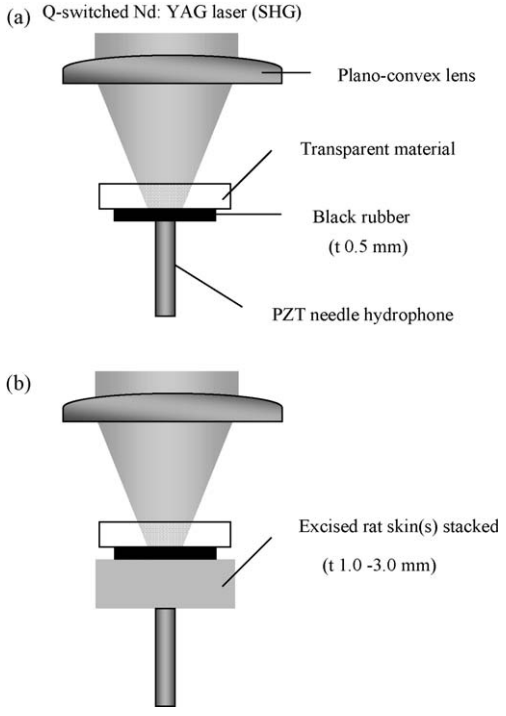

Fig. 2. The experimental setups for measurement of temporal profiles of LISWs.