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Observation of vasculature alternation by intense pulsed light combined with physicochemical methods

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ABSTRACT

Intense pulsed light (IPL) with low energy insufficient to completely destroy a vasculature was applied to rabbit ears to investigate vasculature alteration. Glycerol was combined with IPL to enhance the transfer efficacy of IPL energy. Both trans-illumination and laser speckle contrast images were obtained and analyzed after treatment. The application of IPL and glycerol combination induced vasodilation and improvement in blood flow. Moreover, such phenomenon was maintained over time. IPL may be applied to treat blood circulatory diseases by inducing vasodilation and to improve blood flow.

Introduction

The circulatory system is responsible for delivering oxygen and nutrients to cells and for filtering out waste and carbon dioxide. Malfunction of this system results in diseases, causing a wide range of health problems, many of which can be fatal over time (Pittman, 2011).

Circulatory diseases mostly related to blood flow can be classified into five groups (traumatic, compressive, occlusive, tumors/malformations, and vasospastic) and may occur more commonly in diseases such as diabetes, hypertension, or kidney failure or in dialysis patients (Phillips and Murphy, 2002; Van Buren and Toto, 2011). Further, occupational exposure (vibrating tools and cold) and smoking can cause circulatory diseases. Circulatory diseases have been treated by various therapeutic methods (Dolibog et al., 2014; Games and Sefton, 2013; Pratt, 2010a; Pratt, 2010b), which induce vasodilation to improve blood flow. However, most of the present therapeutic methods are based on the application of drugs, and side-effects have been reported (Yang and Agarwal, 2011). As an alternative to conventional methods, this study introduces the feasibility of intense pulsed light (IPL) in the treatment of circulatory diseases.

IPL has been widely used to treat cosmetic problems, including irregular pigmentation, vascular lesions, telangiectasias, hypertrichosis, and rhytides (Babilas et al., 2010; Goldberg, 2012). The core technology involves the use of a polychromatic broadband flashlamp equipped with optical filters that allow transmission of noncoherent light to the skin in the visible to infrared wavelength range (500 to 1200 nm) (Babilas et al., 2010). The treatment mechanism of vascular lesion is related to the selective absorption of photon energy by endogenous or exogenous chromophores in tissue and the energy transfer to chromophores, such as oxyhemoglobin (predominately found in clinically red lesion), deoxyhemoglobin (predominately found in blue lesion), and methemoglobin, with absorption wavelength peaks of 418, 542, and 577 nm (Goldberg, 2012). The photon energy transfer generates heat and subsequently destroys the target structure (Soltes, 2010). The photon energy transferred from the IPL to the vasculature may act as a stimulant unless the photon energy is sufficiently high to destroy the vasculature. In practice, high IPL energy is used to treat vascular lesion because most photon energy is absorbed by the skin surface region and therefore results in side-effects such as skin surface burn (Goldberg, 2012).

Optical tissue clearing (OTC) is a well known method to make biological tissue transparent and consequently enhance the photon density delivery by reducing the light scattering property in tissue. Microneedling method produces artificial channels in tissue and can reduce the diffusion time of topically applied OTC agent, resulting in faster OTC effect (Yoon et al., 2008).

In this study, low-energy IPL, which is insufficient to destroy the vasculature, was applied to rabbit ears to investigate the efficacy of vasodilation in the vasculature. An OTC agent, glycerol, was combined with IPL to enhance the transfer efficacy of photon energy by

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minimizing light scattering in tissue. Alternation of the vasculature was observed using a laser speckle contrast image (LSCI) and trans-illumination image (TII).

Materials and methods

Animal preparation

Nine ears from five female New Zealand white rabbits (aged 7–8 weeks) were selected and divided into two groups: five ears for the IPL-applied group and four ears for the microneedling-applied group (MAG). The IPL-applied group was divided into two groups: IPL-only-applied group (IOAG) and IPL-applied group with glycerol (IAGG) by selecting different regions. In IAGG, microneedling was applied with a glycerol to reduce diffusion time. The microneedling can be regarded as a physical stimulation to vasculature. Thus, MAG was compared with IPL-applied groups to investigate that the physical stimulation can affect to blood flow or vasculature change or not. The rabbits were intraperitoneally anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) in all image acquisition processes. The hair around the region of interest was shaved before image acquisition. A period of time was reserved for image acquisition to avoid unnecessary stimulation of the vasculature due to shaving. The experimental protocol was approved by the Institutional Animal Care and Use Committees at Yonsei University, Korea.

Optical imaging modalities

When there is blood flow, red blood cells (RBCs) act as moving particles and LSCI can be used to measure the relative flow velocity of the moving RBCs which can be expressed as speckle contrast ranging from 0 (high velocity) to 1 (low velocity). The speckle contrast was converted to speckle flow index (SFI) to observe blood flow. Fig. 1 shows the schematic diagram of the laser speckle imaging modality. A diode laser (HL6512MG; 658 nm, 50 mW; Thorlabs, Newton, NJ, USA) was coupled into an optical fiber. A laser beam passes through a holographic diffuser for even illumination and was directed to a 1:1 beam splitter to eliminate shade in the animal sample. The rabbit ear was imaged with a charge coupled device camera (XC-HR57; Sony, Tokyo, Japan). The imaging area was approximately 25×30 mm^2, and images were acquired at 30 Hz using a frame grabber (DOMINO IOTA; Euresys, Angleur, Belgium).

Fig. 2 shows the schematic diagram of the trans-illumination imaging modality used to evaluate the morphological change in the vasculature. It consists of a DSLR camera (EOS 600D; Cannon, Tokyo, Japan) to obtain color images and a LED ring light as a light source. To eliminate surface specular reflection, two linear polarizers were placed in front of the camera and the ring light at a perpendicular direction. The sample stage was designed to be transparent to pass light. The imaging area was approximately 30×50 mm^2.

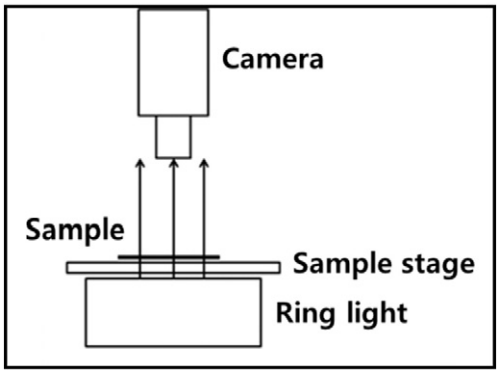

Fig. 2. Schematic diagram of the trans-illumination imaging modality.

Experiment procedure

A 70% glycerol solution was used in conjunction with a microneedle roller (MTS, Clinical Resolution Laboratory, Brea, CA, USA), which was used to reduce the diffusion time of glycerol. An IPL device (Nymph Light, UMES, Wonju, Korea) was used to stimulate the vasculature with the following parameters: 60 J/cm^2, total irradiation time of 24 ms (8 ms $\times$ 3 pulses) with three pulse modes (8 ms pulse duration and 5 ms pulse delay) at an IPL application [Fig. 3], and a 500 nm high pass filter.

The initial state in which no stimulation was given was imaged as a control group. For the MAG, images were acquired after the sample was treated with a microneedle roller. For the IOAG, images were acquired after the sample was treated with IPL. For the IAGG, images were acquired 30 min after the sample was treated with both microneedling and glycerol. Then, the sample was treated with IPL and images were acquired. Both TIs and LSCIs were acquired 1, 6, and 11 days after each treatment in each group. The imaging position was maintained as much as possible to avoid position-dependent artifacts. The camera parameter and surrounding lighting were maintained at the same condition for every image acquisition procedure to ensure image quality.

Results

Fig. 4 shows the TIs and LSCIs of the MAG. The vasculature appeared to be damaged after microneedling application to the sample, presenting vasculature disruption and blood clot as indicated by black and white triangles, respectively [Fig. 4(b)]. The blood clot somewhat decreased in size, and the small vasculature was not clearly observed

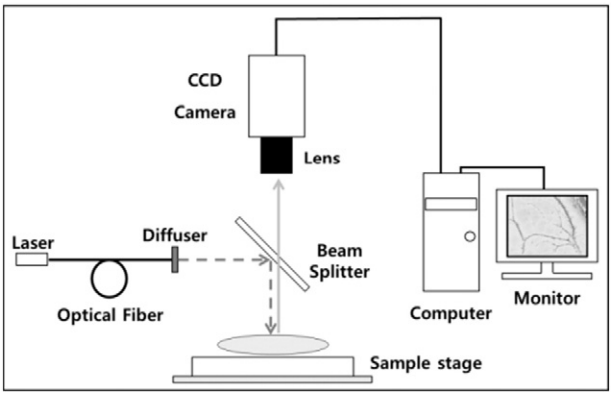

Fig. 1. Schematic diagram of the laser speckle imaging modality.

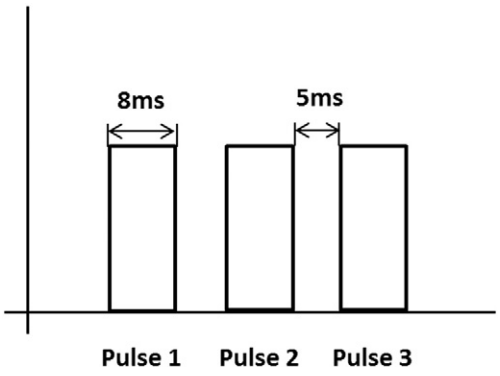

Fig. 3. Temporal pulse train of three pulse mode of IPL application.