INTRODUCTION

Despite the recent advancement in emergency and critical care medicine, there remain many problems in treating severe burn injuries; for improving the outcome of treatment, a method for noninvasive, real-time diagnosis of the wound status is required. One crucial factor for wound management is injury depth; differentiation of superficial dermal burns (SDBs) and deep dermal burns (DDBs) and differentiation of DDBs and deep burns (DBs, also called full-thickness burns) are very important for planning appropriate treatment. However, conventional methods, such as visual observation and pinprick tests, are often inaccurate. Although the usefulness of various optical diagnoses, including laser Doppler imaging, fluorescence imaging, and near-infrared reflectance imaging, has been investigated, these imaging techniques do not provide sufficient quantitative information on injuries. Recently, polarization-sensitive optical coherence tomography (OCT) has been successfully applied to quantitative assessment of burn depth, but its measurement depth is limited to around 1 mm, which is not sufficient for clinical application.

We have been developing a new method for burn diagnosis that is based on the measurement of photoacoustic (PA) signals originating from blood in the wound. The principle of diagnosis is...
described in the next section. The advantages of this method include the ease with which quantitative depth information can be obtained and greater sampling depth compared with OCT-based techniques. The method is also useful for monitoring the wound healing process; time-dependent variation of blood-originating PA signals indicates recovery of blood perfusion in injured tissue. In addition, multiwavelength PA measurement can provide information on local hemodynamics, e.g., hyperdynamic state, which is a unique symptom observed after the shock phase in severe burn cases.  

In this paper, the principle and the device used for multifunctional PA diagnosis of burn injuries are described and experimental data are presented. All data shown here were obtained in experiments using rat models, but the applicability of our method to thick (~3 mm) burns was examined by an experiment using a thick burn mimicking model, and the results of that experiment are also presented.

**PRINCIPLE**

When biological tissue is irradiated with a short light pulse, the light is absorbed by chromophores in the tissue. If the pulse width is shorter than the heat diffusion time, an adiabatic temperature rise is induced in the chromophores, resulting in the emission of a photoacoustic (PA) wave through a thermoelastic process. The PA wave is propagated to the tissue surface; its propagation time provides information on the depth of the chromophores, while its amplitude correlates with the absorption coefficient of chromophores. Thus, depth-resolved distribution of the chromophores can be obtained by PA measurement.

Vascular occlusion occurs in burned skin tissue, while there is abundant blood flow in uninjured tissue located under the wound. Therefore, by irradiating the wound with pulsed light that is selectively absorbed by blood, the light pulse can efficiently propagate through the damaged tissue layer and is then absorbed by blood in the uninjured tissue layer, resulting in the emission of PA waves. The PA waves can be detected with a transducer placed on the wound surface, and their propagation time provides information on the depth of injury (Fig. 1). A conventional piezoelectric element can be used for detecting PA waves. Characteristics of PA waves are similar to those of ultrasound, and they can therefore propagate in tissue much more efficiently than light can. This is the reason why greater measurement depth can be obtained with this method than with pure optical diagnosis methods.

**Figure 2** shows the absorption spectrum of oxy- and deoxyhemoglobin in the visible spectral region;
both hemoglobins show high absorption coefficients from the green to yellow spectral region. This indicates that PA signals originating from blood can be efficiently generated by irradiation with light pulses in this spectral region. Since pulse width of nanoseconds satisfies the requirement for efficient thermoelastic process described above, conventional Q-switched solid-state lasers can be used as the light source.

MATERIALS AND METHODS

Burn Models

All procedures in this study were performed in accordance with the Guide for Laboratory Animal Facilities and Care Regulation of the National Defense Medical College, Saitama, Japan. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg animal weight), and their dorsal hair was clipped and depilated with a hair removal cream. Dorsal skins of rats were exposed for 10 seconds to water heated to 70°C, 78°C and 98°C through a Walker-Mason template to induce a superficial dermal burn (SDB), a deep dermal burn (DDB) and a deep burn (DB) respectively. The area of each burn was approximately 20% of the total body surface area, serving as extensive burn models. All burned rats were resuscitated with saline (25 ml/kg animal weight) immediately after exposure. Histologies of the wounds in these models are given in Reference 13.

Experimental Setup

Figure 3 shows a diagram of the experimental setup
(left) and the configuration of a transducer (right) used for PA measurements. The transducer consisted of an annular P(VdF/TrFE) (vinylydene fluoride trifluoroethylene copolymer) film for the detection of PA waves and a quartz fiber of 600 µm in core diameter for the delivery of light; the film and fiber were coaxially arranged. An optically clear PMMA (polymethyl methacrylate) disk of 6 mm in thickness was attached to the front side of the film in order to reduce the effect of back-scattered light from the samples and to control the acoustic reflection noise generated in the transducer. A tunable coherent light source, an OPO (optical parametric oscillator) was used as a light source for PA excitation; however, the second harmonics (532-nm) of a Q-switched Nd:YAG laser can also be used for simple burn depth measurement. The nanosecond (4-6 ns) output pulses were coupled to the fiber and then used to irradiate the skin to excite PA signal waves. The OPO was operated at a constant repetition rate of 30 Hz, and the pulse energy for irradiation was usually adjusted to 100 ±10 mJ/pulse with a variable attenuator. It was confirmed that the light

Fig. 4 Photoacoustic signals for control (healthy) skin (A), SDB (B), DDB (C), and DB (D) in rats at 6 h postburn. The wavelength was 550 nm. White and black arrows indicate the signal rise point and the peak point, respectively. 13)
fluence on the tissue was much lower than the threshold of light-induced damage for skin. The bandwidth of the transducer (-6 dB levels) was 13.6-39.2 MHz. The transducer signals were amplified by using an FET preamplifier with a bandwidth of 300 Hz - 100 MHz and were recorded in a 300-MHz-bandwidth digital oscilloscope.

Measurement of Photoacoustic Signals

During PA measurements, rats were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg animal weight) and an ultrasonic couplant was used for the interface between the transducer and the wound surface to reduce the reflection loss for the PA signals. For one measurement at each point, PA signals induced by 64 light pulses were averaged and its temporal waveform was displayed in the oscilloscope. Since the light pulse frequency was 30 Hz, the time interval between each data acquisition was 33.3 ms and one measurement took only about 2 s, realizing quasi real-time measurement.

DIAGNOSIS OF BURN DEPTHS

Figure 4 shows typical depth profiles of the PA signals measured at 550 nm for normal skin and SDB, DDB and DB at 6 h postburn; signals of two measurements at two sites (four profiles) for the same rat are shown.

![Graph](image)

**Fig. 5** Relative frequencies of signal peak depth for the control, SDB, DDB, and DB groups at 6 h postburn. The light wavelength was 550 nm.13
Signal rise depth and signal peak depth are indicated by a white arrow and a black arrow, respectively, in each figure. For the normal skin, three prominent peaks were observed in the depth ranges of 0.1-0.2 mm, 0.3-0.4 mm and 0.75-0.85 mm. For SDB, a single peak appeared at a depth of around 0.3 mm. For DDB and DB, two remarkable peaks appeared in each curve; amplitudes of the deeper peaks were higher than those of the peaks in the shallow region. Depths of the higher-amplitude signals for DDB and DB were about 0.7 mm and 1.3 mm, respectively. These data indicate that pronounced peaks in the PA signal shift to a deeper region in the skin as the burn severity increases. Comparison of PA signals with histologies showed that the regions shallower than the first pronounced PA signal peaks coincided with the zone of stasis.

Figure 5 shows relative frequencies of the signal peak depth for control (83 sites, n = 12), SDB (33 sites, n = 7), DDB (41 sites, n = 11) and DB (41 sites, n = 11) groups. The distribution moves to a deeper region as the burn severity increases; there are significant differences in signal rise depths between the control and SDB groups, SDB and DDB groups, and DDB and DB groups (p < 0.001). Differences in signal rise depths are also significant between these groups (p < 0.001). These results suggest that healthy skin, SDB, DDB and DB can be differentiated by the PA signals and, in addition, quantitative information on depth of injuries can be obtained.

All data presented above were obtained by point basis measurement, but by scanning a PA detector and

**Fig. 6** PA tomograms for healthy skin, SDB, DDB, and DB in rats at 12 h postburn. The light wavelength was 550 nm. Arrows show the zone of stasis, i.e., injury depth. 14)
reconstructing signals at each point, 2-dimensional PA signal maps (PA tomograms) can be obtained. Figure 6 shows PA tomograms for 2 cm sections in all burn models at 12 h postburn, where spline interpolation was used for signal reconstruction. The tomograms clearly show the zones of stasis, by which burn depths can be assessed quantitatively.

MONITORING OF WOUND HEALING PROCESS

For management of severe burn injuries, it is important to observe their healing process, which generally has three phases: inflammatory phase, proliferative phase and remodeling phase. After the inflammatory phase, structural changes in injured tissues, including angiogenesis, granulation tissue formation and reepithelialization, occur and these changes can provide information for assessing the wound healing process; angiogenesis can be monitored by measuring PA signals originating from blood in the neovascularities. We detected the time-dependent recovery of blood perfusion in the wounds. In addition, we examined the change in peripheral hemodynamics on the basis of multiwavelength PA measurement.

Figure 7 shows PA tomograms obtained at a wavelength of 532 nm, an isosbestic point for oxy- and deoxyhemoglobins, before and 1.5-120 h after making a DDB in a rat. In each image, signal layer(s) with low to high amplitude was/were observed in specific depth range(s); these signal layers are thought to originate from blood in the tissue. Before injury, three layers with low to medium amplitudes were observed, similar to the control image in Figure 6. At 1.5 h postburn, two signal layers with medium amplitudes were observed; the first signal layer had almost disappeared at 6 h, indicating occlusion of blood flow due to the injury, and the signal amplitude of the second layer

![Fig. 7 Photoacoustic tomograms for a deep dermal burn (DDB) in a rat at various postburn times. The light wavelength was 532 nm.](image-url)
became higher with elapse of time. The second signal layer gradually shifted to a shallower region. Histological analysis showed that the shift of the PA signal peak reflected angiogenesis in the wound. Measurements were also performed for the same model at 576 nm and 600 nm, corresponding to the oxyhemoglobin absorption dominant wavelength and the deoxyhemoglobin absorption dominant wavelength, respectively (see Fig. 2). PA signals at these wavelengths were normalized by the signal at the isosbestic wavelength of 532 nm; the normalized signals reflect the concentrations of oxy- and deoxyhemoglobins in the tissue.

Figure 8 shows the normalized signals at 576 nm and 600 nm as a function of postburn time. During the period from 24 to 48 h postburn, the signal at 600 nm decreased drastically, indicating that the concentration of deoxyhemoglobin decreased in this period. It is known that when passing through the shock phase, hemodynamics shifts to that in a hyperdynamic state; in this state, oxygen consumption associated with hypermetabolism increases, and cardiac output abnormally increased to maintain the increases oxygen consumption. However, oxygen delivery to peripheral tissues is inadequate because oxygen intake is blocked by edema, leading to decrease in the level of deoxyhemoglobin. Thus, hyperdynamic state is an indicator of the end of the shock phase. These findings demonstrate the usefulness of PA measurement for monitoring the healing process of severe burn injuries.

**APPLICABILITY TO DIAGNOSIS OF THICK BURNS**

The thickness of rat skin is typically 0.6 – 1.0 mm, while the maximum thickness of adult human skin reaches > 3 mm. Thus, it should be confirmed experimentally whether blood in uninjured tissue under thick burned tissue can be detected by PA measurement; generally, burned tissues show much higher scattering loss than do healthy tissues. We made a DB (full-thickness burn) in the dorsal skin in a rat and an excised full-thickness burned skin taken from another rat was placed on the wound, the total thickness of burned tissue being ~ 3 mm (Fig. 9 (a)). With this thick burn mimicking model, we attempted to measure PA signals originating from blood in the uninjured tissue under the burned skin tissues. To obtain the signals, light pulse energy was increased to 1 mJ at 576 nm and light reflection from the wound surface was reduced by modifying a plastic spacer in front of the sensor film; light reflection caused noise due to the pyroelectricity of the sensor material.

Figure 9 (b) shows the PA tomogram obtained for the thick burn mimicking model. Although two straight horizontal lines due to artifacts appeared in the shallow region, PA signal layer originating from blood in the native uninjured tissue was clearly shown, suggesting that the present method can be applied to burns of human skin. Before a clinical trial, however, we plan to perform experiments using porcine burn models; porcine skin has close anatomical resemblance to human skin.

**SUMMARY AND FUTURE PROSPECT**

In summary, we have demonstrated the validity of photoacoustic diagnosis of injury depth and local hemodynamics in burns by experiments using rat models. In addition, the result of an experiment using a thick burn mimicking model suggested that the present method can be applied to burns of human skin. It should be noted that simultaneous measurement of both wound depth and hemodynamics is feasible with the same device. Furthermore, although not dealt with in this paper, this technique can also be applied to sensitive monitoring of adhesion of grafted skins. Thus, it is concluded that photoacoustic measurement provides a versatile diagnostic tool for treatment and management of severe burn injuries.
REFERENCES