In Vivo Monitoring of Neovascularization in Tumour Angiogenesis by Photoacoustic Tomography *

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Photoacoustic tomography (PAT) is presented to in vivo monitor neovascularization in tumour angiogenesis with high resolution and high contrast images in a rat. With a circular scan system, the photoacoustic signal, generated by laser pulses at a wavelength of 532 nm from a Q-switched Nd:YAG laser, is captured by a hydrophone with a diameter of 1 mm and a sensitivity of 850 nV/Pa. The vascular structure around the rat tumour is imaged clearly, with optimal contrast, because blood has strong absorption near this wavelength. Serial noninvasive photoacoustic images of neovascularization in tumour angiogenesis are also obtained consecutively from a growing tumour implanted under the skin of a rat over a period of two weeks. This work demonstrates that PAT can potentially provide a powerful tool for tumour angiogenesis detection in cancer research. It will bring us closer to clinical applications for tumour diagnosis and treatment monitoring.

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Studies in oncology have shown that angiogenesis, which refers to the formation of new blood vessels within a tumour or the growth of blood vessels between a tumour and its surrounding tissues, plays an important role in tumour growth and metastasis.[1] It is necessary to develop noninvasive and quantitative tools for monitoring neovascularization in tumour angiogenesis. Traditional histological microvessel density counts[2] and intravital microscopy,[3] lack some of these features. So far, there have been several noninvasive methods for assessing neovascularization in tumour angiogenesis. However, they all have different limitations. Positron emission tomography (PET) scanning often involves contrast agents.[4] Methods based on magnetic resonance imaging (MRI) can provide spatial maps of vessels, while they are limited in temporal and spatial resolutions.[5]

A new noninvasive technique named photoacoustic imaging, combining the advantages of both ultrasound imaging and optical imaging, can provide high ultrasonic resolution and high optical contrast tissue images.[6−9] In recent years, photoacoustic and thermoacoustic imaging has become a popular research subject.[9,10] Photoacoustic tomography (PAT) is based on the measurement of laser-induced ultrasonic waves. The laser-induced ultrasonic signals from a biological sample depend on the optical absorption in the sample to reveal the structure of the tissues based on optical contrast.[11−14] Under illumination by green laser light at a wavelength of 532 nm, the optical absorption of whole blood is much stronger than that of other tissues. Therefore blood generates strong photoacoustic signals and manifests high image contrast, causing the vasculature in organs to stand out prominently in photoacoustic images. Besides optical absorption, the thermal and elastic properties of the tissues also affect the strength of photoacoustic signals. Physical changes, such as density in a tissue, can be reflected in photoacoustic images. The increased density of tumour-bearing tissues is most likely due to a passive mechanism referred to as the enhanced permeability and retention effect.[15] Thus PAT is potentially to evaluate vasculature developing from the early stage of tumour growth.

In this study, we develop a unique photoacoustic imaging system to detect tumour neovascularization associated with angiogenesis in a rat animal model. In this system, a laser pulse at a wavelength of 532 nm serves as the irradiation source, a transducer is used to receive the photoacoustic signal. The vasculature around a tumour underneath the dermis is imaged clearly. We also present images of tumour neovascularization obtained over a two-week period after subcutaneous inoculation of gliosarcoma tumour cells in a rat.

The experimental system for photoacoustic imaging is shown in Fig. 1. A Q-switched Nd:YAG laser (LOTIS TII Ltd, Minsk, Belarus) was used to provide 532-nm laser pulses with a full-width half-maximum (FWHM) value of 6.5 ns, with a repetition rate of 10 Hz. The laser beam was expanded and homogenized to provide an incident energy density of <

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$10\,mJ/cm^2$ on the surface of the rat skin. The diameter of laser beam on the surface of the rat skin is 2.5 cm. It was used for generating the photoacoustic signal during photoacoustic imaging. The hydrophone (Precision Acoustics Ltd, Dorchester, UK) for recording the optoacoustic signals has a diameter of 1 mm and a sensitivity of 850 nV/Pa. The transducer, driven by a computer-controlled step motor to scan around rat tumour, detected the photoacoustic signals in the imaging plane at each scanning position. An amplifier received the signals from the transducer and transmitted the amplified signals to a digital oscilloscope. One set of data at 200 different positions was taken when the receiver moved over 360°. After 200 series of data were recorded, the photoacoustic image was reconstructed with the filtered back projection algorithm.[12] For coupling the photoacoustic signals from the sample to the ultrasonic transducer, the transducers are immersed in a tank of water. The rat back is allowed to protrude into the water tank through a hole in the bottom of the tank and is insulated from the water by a piece of clear polyethylene membrane covering the hole.

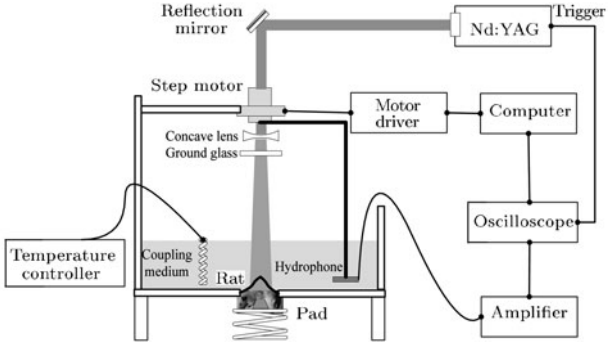

Fig. 1. Experimental setup for photoacoustic imaging.

A rat tumour model was built in our experiment. A rat gliosarcoma cell line, 9L, was obtained from the Shanghai Cell Bank of Chinese Academy of Sciences. Cells were maintained in a 1:1 mixture of Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum (Invitrogen, New Zealand) and 1 mg/ml puromycin (Gibco BRL). The cells were cultured at 37°C in humidified air with 5% CO_2. Microscopy images (magnification ×100, oil immersion) of rat 9L gliosarcoma cells are shown in Fig. 2. The resultant cell clusters (consisting of approximately 10^6 cells) were subcutaneously injected in back of Sprague Dawley rats. The rats were purchased from the Medical School of Jinan University (Guangzhou, China).

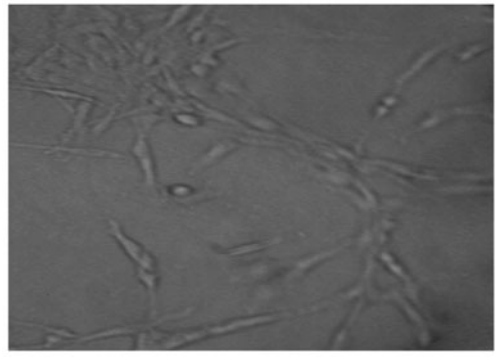

Fig. 2. Microscopy images (magnification 100, oil immersion) of 9 L gliosarcoma cells.

Photoacoustic scanning was performed on day 13 of one rat tumour, as is shown in Fig. 3(a). General anesthesia is administered to the rat by an intramuscular injection of a mixture of ketamine hydrochloride (44 mg/kg), xylazine hydrochloride (2.5 mg/kg), acepromazine maleate (0.75 mg/kg), and atropine (0.025 mg/kg).

In order to observe vascularization in tumour development, serial photoacoustic imaging was carried out in two weeks on day 5, 8, 11, 14 of tumour cell inoculation. An area of approximately 2 cm × 2 cm

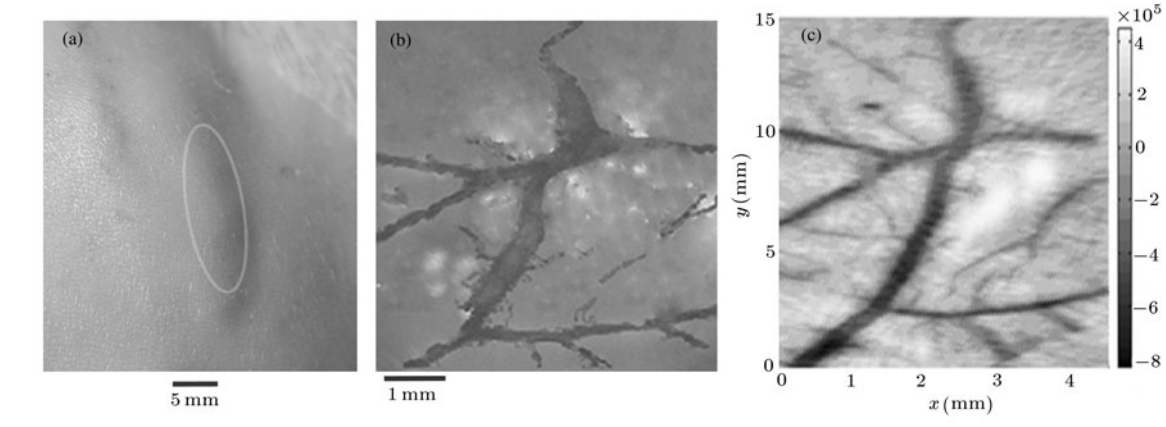

Fig. 3. An *in vivo* photoacoustic image of tumour on a living rat back. (a) Photograph of the tumour on rat back. The tumour area was marked by an elliptical line. (b) An open-skin photograph of the tumour. (c) Photoacoustic image of the rat tumour.

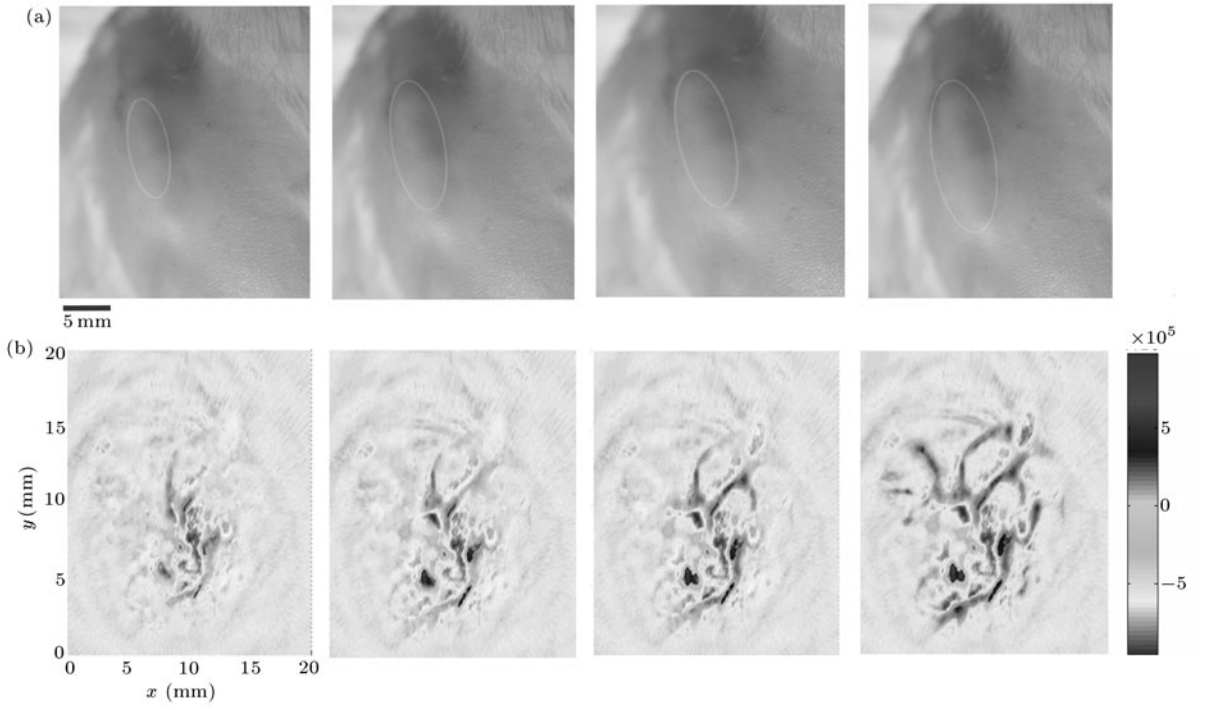

Fig. 4. Several photoacoustic images obtained during the incubation period between day 5 and day 14. (a) Photographs of the tumour on a living rat back on days of 5, 8, 11, and 14 (from left to right). (b) Serial photoacoustic images obtained to observe tumour proliferation during the incubation period between day 5 and day 14.

around the inoculation site is defined by a marker to locate the same spot for serial scanning (the photograph are shown in Fig. 4(a)).

Figure 3 presents an *in vivo* photoacoustic image of tumour on a living rat back. Figure 3(a) shows a photograph of the rat back tumour; the tumour area is marked by an elliptical line. In Fig. 3(b), an open-skin photograph shows the tumour clearly on the rat back, which illustrates abundant tortuous irregular distorted vessels around the tumour. In Fig. 3(c), the tumour associated vasculature distortion can easily be recognized on the photoacoustic image. As we can see, in the tumour area, the normal vascular architecture is seriously distorted by the growing new blood vessels. The close-up image of the tumour area clearly shows the vascular distortion inside and around the tumour. The abnormal vessels that are owing to angiogenesis surround and feed the tumour. From the experimental results, it is demonstrated that the tumoral vascular structure can be clearly imaged noninvasively by PAT.

Because photoacoustic imaging is non-invasive, tumour neovascularization can be monitored without sacrificing the animal, which enables serial measurements in the same animals. Several photoacoustic images were obtained during the incubation period between day 5 and day 14. These images reveal the process of early tumour proliferation. Several blood vessels were seen in the photoacoustic image on day 5, suggesting a tumour mass surrounded by developing vessels (Fig. 4(a)). The series of images reveal a major increase in photoacoustic response in the period between day 5 and day 14 (Figs. 4(b)–4(d)). The vessel density has noticeably increased and some vessels have increased in size, suggesting a tumour mass surrounded by developing vessels.

In summary, we have demonstrated that photoacoustic imaging is a noninvasive means for localizing and quantifying the vessels around tumour with high optical contrast and high ultrasonic resolution *in vivo*. In our experiment, the spatial resolution of the imaging system is estimated according to the Rayleigh criterion, and the spatial resolution is determined to be 60 μm.[16] This feature makes photoacoustic imaging a promising new tool in tumour angiogenesis research. At present, our photoacoustic system involves only a single-element ultrasonic transducer. The total acquisition time for one photoacoustic time trace, consisting of 32 averages and including the data transfer from the oscilloscope to the computer, is about 4 s at each position. For a 2π angular scan with a step size of 1.8°, the image acquisition time is about 15 min. In the future, the reduction of the scanning time will be realized by using an array of simultaneously sampled acoustic detectors and a laser with a higher pulse rate.[17,18] Furthermore, non-invasive assessment of the extent of tumour vasculature may become of importance in clinical evaluation of the tumour and monitoring therapy based on angiogenesis inhibition. The measurement strategy followed in our study is not restricted to superficial tumour and can therefore be applied to study

tumour in brain of rat.

Photoacoustic imaging can provide both high resolution and high contrast images to detect rat tumour by imaging of the tumour-related vasculature. This feature makes photoacoustic imaging suit for monitoring tumour growth, angiogenesis, and antiangiogenic therapy in experimental carcinogenesis on animal models.

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