In vivo photoacoustic imaging of osteosarcoma on animal model

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Abstract. Osteosarcoma is the commonest primary malignant tumor of bone, and the second highest cause of cancer-related death in the pediatric age group. Although there are several methods for osteosarcoma detection, e.g. X-ray, CT, MRI and bone scan, they are not satisfied methods because they can hardly detect osteosarcoma in early stage. Photoacoustic imaging (PAI) is an emerging hybrid imaging modality that is noninvasive, nonionizing, with high sensitivity, satisfactory imaging depth and good temporal and spatial resolution. In order to explore this new method to detect osteosarcoma, we established SD rat models with osteosarcoma and utilized PAI to reconstruct the osteosarcoma image in vivo. This is the first time detecting osteosarcoma in vivo using PAI, and the results suggested that PAI has potential clinical application for detecting osteosarcoma in the early stage.

1. Introduction
Osteosarcoma is the prominent primary bone cancer in humans, excluding hemopoietic malignancies. It is usually highly aggressive and eventually lethal, and mainly affects children and adolescents. More than 80% of these tumors tend to occur in the long bones of the appendicular skeleton which are undergoing rapid growth[1]. The age at presentation ranges from 10 to 25 years. Several years ago, all patients with osteosarcoma were treated by amputation but the cure rate was under 10% and almost all patients died within a year from diagnosis. Today, for localized osteosarcoma at onset (80% of cases) treated in specialized bone tumor centres with pre- and postoperative chemotherapy associated with surgery, the percentage of patients cured varies between 60% and 70%. However, in patients with metastasis at onset cure rate is about 30% [2]. Obviously, this great therapeutic effect progress is based on the confirmed diagnosis in the early stage and the tumor is localized osteosarcoma which has not ever migrated to other organs. Early detection and treatment are critical for managing patients with osteosarcoma. Although there are several methods for osteosarcoma
detection in clinic, e.g. plain radiographs, computed tomography, magnetic resonance imaging, angiography, dynamic bone scintigraphy and, if necessary, the type of reconstruction, there is not a satisfied enough method can detect osteosarcoma in the early stage [2-5]. Therefore, it is very necessary to find a new way to detect osteosarcoma, especially at the early stage.

PAI, also referred as photoacoustic tomography (PAT) or optoacoustic tomography, is a new imaging technology. It is a potential candidate for osteosarcoma detection because this hybrid technique combines the high intrinsic optical contrast with the high spatial ultrasound resolution. What’s more, it is non-invasive and non-destructive and does not rely on the use of ionizing radiation[6]. PAI is based on the detection of acoustic waves generated by absorption of pulsed light in tissue. Owing to the initial ultrasonic pressure is proportional to the local optical energy deposition in the tissue, the induced acoustic signals exhibit heterogeneity of optical absorption in tissue and the PAI reveals the optical absorption distribution of tissue. PAI has been applied to in vivo imaging of vasculature in small animals and humans[7-11], and early cancer detection[12-14]. The PA technique has also been used for non-invasive monitoring of blood oxygenation (especially cerebral oxygenation), cerebrovascular activities in small animals [15-17] and monitoring of skin abnormalities [18].

In this study, osteosarcoma models in rats was established. The rat leg was imaged in vivo with PAI. As an essential step toward our ultimate goal, the feasibility of PAI in imaging osteosarcoma in vivo was verified through the study on rats osteosarcoma models.

2. Materials and methods

2.1 Establishment of osteosarcoma rat model
Male Sprague-Dawley rat, 4 weeks old, was purchased from the laboratory animal center of Sun Yat-sen University. The rat was maintained in microisolation cage under specific pathogen-free conditions, in accordance with the institutional guidelines of the South China Normal University Ethics Committee and under the supervision of authorized investigators. The UMR 106 osteosarcoma cell line syngeneic to SD rat was purchased from the American Type Culture Collection (ATCC; Manassas, VA) and cultured in RPMI-10% FBS (Sigma) supplemented with L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml) confirmed to be mycoplasma-free by routine testing. The number of the cells were adjusted to about 2×10⁷ /ml and the rats were anesthetized intraperitoneally with 10% chloral hydrate at a dosage of 0.1 ml/30 g live weight, then the cells were injected directly into the proximal part of the tibia shaft surface of anesthetized SD rat with a syringe and 25 gauge needle. After implantation, the rat was returned to cage and fed for 15 days as before. The rat was treated with cyclosporin A (10 mg/kg) every day until one week after the implantation and the image reconstruction was performed 15 days following the methods as described in detail below [19,20].

2.2 PAI principle and image reconstruction
PA signals are caused by optical energy deposition in target tissue. The strength of the PA signal is proportional to the local absorbed energy density. When the laser pulse is short enough, thermal diffusion can
be ignored, and the resulting PA pressure $p(r,t)$ that reaches a detector at position $r$ and time $t$ can be expressed as [21,22],

$$p(r,t) = \frac{I_0 \beta}{4\pi C_p} \int \int \int \int \frac{d^3(r')}{|r-r'|} A(r') T(t')$$

Where $\beta$ is the isobaric volume expansion coefficient, $C_p$ is the specific heat, $I_0$ is a factor proportional to the incident optical energy density, $T(t')$ denotes the temporal profile of the light with units of 1/s, which can be regarded as a Dirac delta function $\delta(t)$ for a short laser pulse, and $A(r')$ is the absorbed optical energy deposition per unit volume of soft tissue at position $r'$. The PA signal propagates from its originating region can be detected by an ultrasonic transducer or an array detector. Through a reconstruction algorithm, the distribution of absorbed optical energy $A(r')$ in a target tissue can be reconstructed from a set of measured PA signals $p(r,t)$. In the current study, a modified filtered back-projection algorithm developed by our early work is used to reconstruct the distribution of absorbed optical energy density [23].

2.3 Experimental setup

A PAI prototype system was employed in this study, as shown in Fig. 1 A Q-Switched Nd: YAG laser (LOTIS TII Ltd, Minsk, Belarus) is used as excitation source, which operates at 1064 nm with pulse duration of 10 ns and pulse repetition rate of 15 Hz. The laser beam was expanded by a concave lens and then illuminated into the rat leg (diameter of the beam is approximately 1.3 cm). The incident energy density on the leg surface was set below 20 mJ/cm². PA signals are detected by a 64-element linear transducer array. Each element of the array has a width of 0.3 mm, a height of 4 mm, and a center frequency of 7.5 MHz with a 70% bandwidth. A custom-built water tank coupled the PA signals between the transducer array and the mouse leg. The mouse was fixed in a box, and the box was controlled by a precise stepper motor to scan circularly around its axial in the horizontal plane X-Y plane. To cover a 360° receiving angle for imaging the mouse leg, a total of 20 steps with a constant 18° interval were taken. The PA signals acquired from the transducer array were transmitted by a custom 64-ch acquisition system to the personal computer, and then processed by the reconstructed program. The ultrasound speed was assumed to be exactly 1500 m/s for every PA reconstructed image.
2.4 PAI of rat leg in vivo
The rat was anesthetized using the same method as previously mentioned and fixed on the experiment instrument as shown in the Figure 1. The PAI image of horizontal cross section of rat leg with osteosarcoma, at the proximal end of tibia, was reconstructed using the same PAI methods at 15 days after the rat was injected the osteosarcoma cells.

2.5 Histopathological examination
After the PAI image was reconstructed, the rat leg with tumor was cut down and saved in 10% buffered formalin for 2 days. The leg was then decalcified with formic acid for 4 days and monitored with a Faxitron MX-20 x-ray machine. Once decalcification was completed, the specimen was dehydrated with graded alcohol, embedded in paraffin, cut into blocks, and sectioned to 7-μm thickness with a Reichert-Jung 20/30 metal knife. Hematoxylin and eosin staining of specimen sections on glassslides was conducted. Last, histological pictures of specimen section was taken with a 10×magnification.

3. Results

3.1 PAI of sick rat leg in vivo
The PAI of the sick rat leg 15 days after injected UMR-106 osteosarcoma cells, is shown in Fig. 2(a). Compared with its transverse section anatomical photograph Figure 2(b), the tumor was unequivocally detected with PAI and matched well both in position and shape. The images suggest that PAI not only can detect the osteosarcoma, but also can distinguish the range of the tumor. The images from PAI provide the information on tumor changes in addition to osteosarcoma tissue in the leg.
**Figure 2.** Noninvasive PAI images of sick rat leg in vivo. (a) PAI image on 15 days after injected tumor cells; (b) Histological photograph of a similar cross section in the sick rat leg on 15 days after PAI was finished.

3.2 Histopathological evaluation

The rat was submitted to necropsy after experiment as described before. (Fig. 2) Cut the tissue corresponded the region with increased optical absorption. The specimen was analysis by routine pathology and definited the tissue was osteosarcoma. (Figure 3)

**Figure 3.** Histopathological test with hematoxylin-eosin staining demonstrated an implanted osteosarcoma (× 100). Implanted tumor was characterized by fusiform or triangle cells (arrow) along nutrient vessels with nuclear pleomorphism, mitoses, bone invasion and necrosis.
4. Discussion and conclusion

In the study, the PA image of horizontal cross section of the sick rat leg was achieved in vivo, and the feasibility of osteosarcoma detection with PAI technique has been demonstrated. With the high resolution, PAI can be expanded to help definite the tumor site, evaluate the stage and the developmental trend of the tumor by series of PA images. Therefore, PAI provides a new method for early detection of osteosarcoma.

In our study, the diameter of the rat leg is about 12 mm, and its cross section can be visualized. According to the experiments reported, 30 mm depth PA image can be achieved [24]. While for the osteosarcoma diagnosis on human, 30 mm is not deep enough for most patients. To get a deeper imaging depth, the contrast should be employed in the imaging. With the development of NIR contrast, a new deeper imaging depth can be reached.

To get a full view data, the PA signals of 50 steps with each step of 3.6° were recorded, the scanning time is about 15 minutes. To get a volume data, more time must be expensed, which will hinder the PAI application in osteosarcoma diagnosis in clinic. In the system, the data acquisition time was limited by the scanning process and the repetition of laser. Therefore, transducer plane should be developed for reducing the scanning time. With the development of high repetition of the laser, the data acquisition time can be reduced greatly.

Though some details of cross section of the rat leg can be visualized in the experiment, the resolution is still not good enough for early detection of osteosarcoma. The resolution of the system is limited to the bandwidth of the transducer array. To get a higher resolution, wider bandwidth transducer array should be considered to employ.

In summary, we have first used the noninvasive PAI technology in osteosarcoma diagnosis for imaging osteosarcoma at the early stage and monitoring the osteosarcoma developing. The in vivo imaging results using a rat model suggest that PAI have the feasibility for mapping osteosarcoma and monitoring osteosarcoma developing. Therefore, the PAI technology has the potential to be applied in osteosarcoma diagnosis, especially at the early stage, and the osteosarcoma patients will have a better therapeutic effect with help of PAI technology.

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