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Evaluation of bladder microvasculature with high resolution photoacoustic imaging

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Abstract

We explored the potential of an emerging laser-based technology, photoacoustic imaging (PAI), for bladder cancer diagnosis through high resolution imaging of microvasculature in the bladder tissues. Imaging results from ex vivo canine bladders demonstrated the excellent ability of PAI in mapping three-dimensional microvasculature in optically scattering bladder tissues. By comparing the results from human bladder specimens affected by cancer to those from the normal control, the feasibility of PAI in differentiate malignant from benign bladder tissues was also explored. The distinctive morphometric characteristics of tumor microvasculature can be seen in the images from cancer samples, suggesting that PAI may allow in vivo assessment of neoangiogenesis that is closely associated with bladder cancer generation and progression. By presenting subsurface morphological and physiological information in bladder tissues, PAI, when performed in a similar way as in conventional endoscopy, provides an opportunity for improved diagnosis, staging and treatment guidance of bladder cancer.

Despite the advances in detection and therapy in the past decades, bladder cancer remains an important public health problem, with no improvements in incidence or associated mortality since 1975 [1]. Urethrocystoscopy (UCS) is the current gold stand in diagnosis and follow-up of non-muscle invasive bladder cancer. However, as an en face imaging modality, UCS cannot render depth resolution and 3D structural information of a tumor; hence, the discriminating between flat carcinoma in situ (CIS) and inflammation is challenging [2].

Tumor stage, histologic grade, and regional lymph node status are currently used to obtain prognostic information about bladder cancers. Researchers are still looking for additional functional or molecular biomarkers that can significantly improve the sensitivity and specificity for early diagnosis, objective grading of tumor and accurate monitoring of therapeutic effects. A majority of studies have been focused on measuring tumor neoangiogenesis. Extensive clinical and preclinical research have demonstrated that neoangiogenesis, playing a crucial role in cancer generation and progression, is a prognostic marker and a potential target for therapy of bladder cancer [3]. As indicated by a large number of studies, neoangiogenesis can be quantified by many morphometric characteristics including microvascular density.

Photoacoustic imaging (PAI), as an emerging optical modality, holds promise for diagnostic imaging and guided therapy of bladder cancer. Unlike photodynamic diagnosis (PDD) that

detects the fluorescent light [4] or optical coherence tomography (OCT) that measures the light reflectance [5], both explored for improved detection of bladder cancer, PAI demonstrates different optical absorption contrast in bladder tissues. Due to the strong intrinsic optical absorption of hemoglobin, PAI is extremely sensitive in mapping microvasculature, and could objectively evaluate the neoangiogenesis that is closely associated with metastasis, recurrence, grade, and mortality of bladder cancer [6]. With the high-resolution 3D map of microvasculature presented in a PAI image, morphometric characteristics of bladder tumors over normal bladder tissues, including enhanced microvascular density, elevated complexity of vascular network and changes in size and shape of microvessels, could be quantitatively assessed. By using multiple laser wavelengths, spectroscopic PAI may further facilitates direct and sensitivity assessment of decreased blood oxygen saturation presenting the regional hypoxia which is another hallmark of bladder cancer [7].

In this work, through the experiments on both canine and human bladder specimens, the performance of PAI in mapping microvasculature in bladder tissue was studied for the first time. By comparing the results from human bladder specimens affected by cancer to those from the normal control, the feasibility of PAI in differentiate malignant from benign bladder tissues was also explored.

Figure 1(a) presents the schematic of a microscopic PAI setup for imaging of microvasculature in a bladder. The Nd:YAG laser (Spot-10-200-532, Elforlight Ltd, UK) working at 532 nm has a pulse duration of 2ns and a repetition rate (PRR) of 0–50 KHz. The laser light was spatially filtered by an iris and then expanded to a parallel beam which was rastered over the tissue object by 2D Galvanometers. The intensity and the stability of the laser beam was monitored and calibrated by a photodiode (DET10A, Thorlabs, NJ). An achromatic lens with a focal length of 50 mm was used as the objective lens. Photoacoustic signals were detected by a calibrated needle hydrophone (HNC-1500, Onda, CA) with –10 dB bandwidth of 300 kHz–20 MHz. The distance between the hydrophone and the tissue was 5 mm, and the ultrasound coupling was through water. The detected photoacoustic signals, after a low noise amplifier (AH 2010, Onda, CA), was digitized by an A/D card (Razor CS14X2, GaGe, IL). The spatial resolution of this system was measured by imaging an USAF resolution template (T-20-P-TM, Applied Image Inc, NY). Determined by the optical focusing, the lateral resolution was 5 μ m. Limited by the central frequency and bandwidth of the hydrophone, the axial resolution of this system was 105 μ m.

To examine the performance of PAI in mapping the microvasculature in bladder tissues, an experiment was conducted on whole canine bladders that were harvested immediately after the animals were euthanized. Bladders from adult dogs provide a good sample to validate PAI of human bladders due to their morphological and histological similarities. Moreover, canine bladder cancer shares morphologic, molecular and clinical features with human bladder cancer. To image the inner bladder wall, as the situation in UCS, the bladder was open using a scalpel and the inner surface of the bladder was exposed. The acquisition of each 3D image was taken through a raster of 256 $\times$ 256 steps along the tissue surface.

An example PAI imaging result from a fresh canine bladder specimen was presented in Fig. 2(a). In this maximum amplitude projection (MAP) image, multiple orders of branching architecture from large vessel to capillary network can be clearly discerned with excellent contrast and continuity. With satisfactory lateral resolution, PAI can realize 3D tomographic imaging of subsurface tissues and retrieve the microvascular structure with depth sectioning ability. As shown in Fig. 2(b), the depth-encoded MAP image [8] of a canine bladder specimen presents distinctive vascular structures at different depths. The vessels at superficial layer are mainly dense capillary network; while subsurface tissues at 488 μ m

depth contain mostly large vessels with less capillaries. As a result of the high sensitivity of PAI to hemoglobin, smallest capillaries with sizes around 5 μm and even individual red blood cells can be imaged clearly based on endogenous contrast without need to involve contrast agents [9, 10].

A preliminary study has also been completed to explore the potential of PAI to differentiate the malignant bladder tissue from the normal based on the morphometric characteristics of microvasculature in tumors. Human bladder specimens from transurethral resection were employed in this study. Based on the results from histopathological examination, these tissues are either normal or contain malignancy, which will then be used as a gold standard to interpret findings from PAI images. Example images from normal and malignant human bladder tissue specimens are shown in Fig. 3. In the image from the normal tissue, vascular structures from large vessels to capillary network can all be recognized, similar to the results acquired from canine bladder specimens. In the image from the malignant bladder tissue, distinctive microvascular features can be seen, including enhanced microvascular density, elevated complexity of the microvascular network, and changes in size and shape of microvessels. It is expected that these morphological differences in microvascular structures are the consequences of neoangiogenesis which is the essential step in bladder cancer generation and progression [6].

Because the microscopic PAI system is based on optical focusing, the achievable imaging depth is limited by the mean free path of light in targeted biological tissues. According to our estimation as well as experimental measurements on bladder specimens and other optically scattering biological tissues, the achievable imaging depth was approximately 1 mm. This imaging depth is sufficient for the evaluation of non-muscle invasive superficial bladder tumors including CIS. However, for muscle invasive tumors, especially large tumors in stages T2–T4, the ability of imaging on a much larger scale is essential. PAI can overcome the “soft-depth limit” determined by the optical transport mean free path by employing ultrasonic focus instead of optical focus, so it has a unique advantage of achieving high-resolution functional and molecular imaging *in vivo* in optically scattering biological tissues at unprecedented depths[11]. Therefore, we expect that PAI, when conducted in a transurethral manner, could contribute to a broad spectrum of bladder tumors including both superficial and muscle invasive.

The results in this study has suggested that PAI has the excellent capability in 3D mapping of microvasculature in bladder tissues, and holds potential to differentiate malignant tumors from normal tissues based on assessment of morphometric characteristics of the microvasculature in tumors as a result of neoangiogenesis. The laser Spot-10-200-532 has a repetition rate of 0–50 KHz. To acquire one 3D image with 256×256 scanning A-lines, an acquisition time of as short as 2.6 second is needed. For initial screening, the imaging area can be easily scaled up by increasing the range of the driving voltage of the galvanometers, with a sacrifice of spatial resolution. Once suspicious lesions are located, a close-up scan of the target areas can be conducted with a higher resolution. We can also use UCS to screen the larger area of the bladder first before conducting PAI to get high-resolution images, as in the current procedure of transurethral optical coherence tomography (OCT) of bladder cancer [5].

As a preliminary study, this work has several limitations. First, the numbers of specimens involved in the experiments were limited. In the study on human bladder specimens, three normal and three cancer samples were used which were not sufficient to draw any conclusions with statistical significance. Second, all the experiments were conducted on *ex vivo* tissues which, although kept fresh before image acquisition, still were not studied within the context of physiologically authentic environments. Our future work includes

imaging of bladder tumors *in vivo* by employing canine bladder cancer models. With the results from a large number of samples, statistical analyses could then be conducted to find whether PAI, based on quantitative measurements of morphological features (e.g. microvascular density, major/minor axis lengths, area, compactness, shape factor and Feret diameter) and physiological parameters (e.g. blood volume and hypoxia), can differentiate malignant tumors from normal tissues[12–14]. One of the practical limitations of PAI is its limited scanning speed which can be resolved later by using lasers with higher repetition rates. As another practical limitation, miniaturization of the set up is critical for PAI to be applied to bladders in a transurethral way, the recently developed pure optical photoacoustic microscopy based on the optical fiber delivered microring resonator[9] provides a promising solution. A PAI based on optical fibers can potentially be used as a complement to current UCS technology.

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Fig. 1.
(Color online) Schematic of microscopic PAI of microvasculature in a bladder ex vivo

Fig. 2. (Color online) (a) Maximum amplitude projection (MAP) image on x-y plane of the vasculature in a canine bladder specimen. (b) Depth-encoded MAP image of the microvasculature in another canine bladder specimen.

Fig. 3.

(Color online) PAI and histological assay of normal and cancerous human bladder specimens. PAI images of (a) a normal and (b) a tumor specimens. (c) Low magnification and (d) high magnification histological photographs of the normal specimen sectioned down from the area of (a). (e) High magnification histological photograph of the tumor specimen depicting infiltration of large disordered cells into superficial lamina propria (marked by arrows). (f) Another histological photograph of the tumor depicting the loss of polarization and anisocytosis. The inset is the magnification of the marked area presenting the rare mitotic figures. (e) showing papillary neoplasm (mucosal fragment) and (f) showing papillary neoplasm consisting of thick epithelium were sectioned down from the area of (b).