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Different optical spectral characteristics in a necrotic transmissible venereal tumor and a cystic lesion in the same canine prostate observed by triple-band trans-rectal optical tomography under trans-rectal ultrasound guidance

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Abstract
Different optical spectral characteristics were observed in a necrotic transmissible venereal tumor (TVT) and a cystic lesion in the same canine prostate by triple-wavelength trans-rectal optical tomography under trans-rectal ultrasound (TRUS) guidance. The NIR imager acquiring at 705nm, 785nm and 808nm was used to quantify both the total hemoglobin concentration (HbT) and oxygen saturation (StO2) in the prostate. The TVT tumor in the canine prostate as a model of prostate cancer was induced in a 7-year old, 27 kg dog. A 2 mL suspension of 2.5×10^6 cells/mL of homogenized TVT cells recovered from an in vivo subcutaneously propagated TVT tumor in an NOD/SCID mouse were injected in the cranial aspect of the right lobe of the canine prostate. The left lobe of the prostate had a cystic lesion present before TVT inoculation. After the TVT homogenate injection, the prostate was monitored weekly over a 9-week period, using trans-rectal NIR and TRUS in grey-scale and Doppler. A TVT mass within the right lobe developed a necrotic center during the later stages of this study, as the mass presented with substantially increased [HbT] in the periphery, with an area of reduced StO2 less than the area of the mass itself shown on ultrasonography. Conversely, the cystic lesion presented with slightly increased [HbT] in the periphery of the lesion shown on ultrasound with oxygen-reduction inside and in the periphery of the lesion. There was no detectable change of blood flow on Doppler US in the periphery of the cystic lesion. The slightly increased [HbT] in the periphery of the cystic lesion was correlated with intra-lesional hemorrhage upon histopathologic examination.

Keywords: Diffuse optical tomography, transmissible venereal tumor, cyst, hemoglobin, oxygen saturation.

1. INTRODUCTION

Prostate cancer has been the second leading cause of death in American men in recent decades [1]. Digital rectal examination (DRE) and serum testing of prostate specific antigen (PSA)
concentrations are the main screening methods in prostate cancer detection. The patients will be referred to prostate biopsy given an abnormal DRE result or PSA level. Trans-rectal ultrasound (TRUS) guided needle biopsy is considered an essential procedure in the definitive diagnosis of prostate cancer [2]. However, low sensitivity and specificity of the TRUS imaging between cancerous lesions and normal tissue leads to an overall accuracy much lower than 50% in prostate cancer detection [2]. Diffuse optical tomography using near-infrared (NIR) light was demonstrated as a non-invasive functional imaging technique on breast cancer [3-5] and has been extended to prostate imaging [6]. A trans-rectal NIR imaging system with concurrent TRUS for visualization guidance was reported on in-vivo imaging of canine prostate bearing a transmissible venereal tumor (TVT) [7]. The growth of a TVT tumor in that subject was associated with an increased total hemoglobin concentration [HbT] [8] indicative of angiogenesis, which agrees with the correlation found between the prostate cancer and increased micro-vessel density in another study [9]. There was approximately a 300% increase in [HbT] along a 7-week course of TVT tumor growth [8]. The estimated tumor volume also grew exponentially over the same time period.

In the prostate, changes in local oxygen saturation were observed via interstitial NIR measurement during prostate photodynamic therapy [10]. Tissue oxygenation status is considered an important prognostic indicator for androgen-deprivation therapy of newly diagnosed metastatic prostate cancer [11]. There is evidence that hypoxia exists in the nidus of prostate cancer [12]. The ability of quantifying the hemoglobin concentration and oxygen saturation in prostate tissue is important for the diagnosis, prognosis, and treatment of prostate cancer. Augmented StO2 mapping of prostate is expected to provide more definitive information for cancer diagnosis when combined with [HbT] mapping and Doppler US imaging.

Prostatic cysts are benign lesions in the prostate that are considered congenital or secondary to neoplasia and prostatic hyperplasia/inflammation. Regardless of etiopathogenesis, cysts are typically lined by secretory epithelium. Generally, they are small, well defined structures filled with prostatic fluid, and they can be predisposed for bacterial infection [13]. The appearance of cysts is often an indication of bacterial prostatitis. The ultrasonographic appearance of the prostatic cyst is usually hypo-echoic, remarkably similar in appearance to that of a homogenous cell type tumor. The optical property of an intact prostate cystic lesion, has, to our knowledge, not been previously reported. In breast cancer, some studies showed that cysts could have increased contrast of optical absorption because of the light transmission rather than scattering effects [14]. Recent DOT studies focusing on breast cancer research showed lower imaging contrast of the cyst on both optical absorption and scattering [15]. Also, in several cases cystic lesions were presented with higher contrast of optical absorption and scattering, but the [HbT] and StO2 were reported to be lower than normal tissue [16].

This study reports the difference in the optical contrasts of a solid tumor and a contra-lateral cystic lesion in an intact canine prostate. In this study, a dog with a cystic lesion in prostate was injected with TVT tumor cells and monitored over 9-week duration using a triple-wavelength trans-rectal US-guided optical tomography system. The 3 wavelengths were 705nm, 785nm and 808nm, covering a ~100nm bandwidth. Both [HbT] and StO2 in canine prostate were mapped in situ by optical imaging. Each week the dog was monitored by Doppler US, TRUS and optical imaging. An increased [HbT] associated with the tumor foci was compared with a relatively stable [HbT] in the periphery of the cystic lesion during 9-weeks of tumor development. Differences in StO2 were also found between the tumor and the cyst.
2. MATERIALS AND METHODS

2.1 Triple-band trans-rectal near-infrared optical tomography

Near-infrared light at three wavelengths were applied to a previously demonstrated trans-rectal NIR/US sagittal-imaging system (diagram in Fig. 1-a and photograph in Fig. 1-b) [8]. The NIR source and detector channels were placed laterally, symmetric to the sagittal US transducer to perform volumetric imaging, of which the mid-sagittal NIR plane was co-registered with the sagittal US plane. The outputs from laser diodes of 705nm, 785nm and 808nm (Thorlabs Inc.) were combined by a tri-furcated fiber bundle (FiberTech Optica Inc.), and sequentially delivered to the 7 source channels of NIR applicator via a fiber switch installed on a linear translation stage (Zaber Technology Inc.). Each of the laser diodes of 785nm/100mw and 808nm/200mw was controlled by one turn-key TEC/driver module LDC3722 (ILX lightwave Inc.). The laser diode of 705nm/50mw was controlled by a turn-key TEC/driver LDC205C (Thorlabs, Inc.). The 7 detection channels were coupled to a spectrometer of 300mm focal-length (Acton Research) which covered around 120nm of bandwidth for separating the three bands of remitted light. The acquisition of the spectrally separated light by a 16-bit intensified CCD camera (Princeton Instruments) was synchronized with the sequential source illumination. The total acquisition time for each data set was about 3 seconds. The trans-rectal NIR/US applicator developed based on ALOKA UST 672-5/7.5 biplane prostate probe is illustrated in Fig. 1-a. In the image formation, the absorption coefficients ($\mu_a^1$, $\mu_a^2$ and $\mu_a^3$) were reconstructed first, then oxygenated and deoxygenated hemoglobin concentrations were derived by

$$[C] = \left( \begin{bmatrix} \varepsilon \end{bmatrix}' \begin{bmatrix} \varepsilon \end{bmatrix} \right)^{-1} \begin{bmatrix} \varepsilon \end{bmatrix}' \begin{bmatrix} \mu_a \end{bmatrix}$$

where $[C]$ indicates a concentration vector of oxygenated [HbO] and deoxygenated [Hb] hemoglobin, and $\varepsilon$ denotes the vector of molar absorption coefficient of [HbO] and [Hb].

Figure 1. Triple-band trans-rectal NIR optical tomography system (a) Schematic payout of the system; (b) Photograph of the completed system.

Figure 2 illustrates the results of calibrating the system measurements on [HbT] and StO2. The calibration of [HbT] measurements compensates the underestimation of the [HbT] due to the non-linear reconstruction of diffuse optical tomography [17, 18]. In the calibration process, a tube of
15mm in diameter made of tissue-mimicking phantom material filled with different concentrations of bovine blood was placed in the middle range of the NIR probe-span and on top of the probe. In calibration of the StO2 measurement, sodium dithionite was added to the blood to decrease the oxygenation without destroying the blood cells. The decreased trend of the derived StO2 was recovered linearly, though the range of averaged StO2 values in the region of the blood were much smaller than the actual values estimated from the de-oxygenation model of sodium dithionite [19]. No conversion from the derived StO2 values to the expected ranges was made in this study. Therefore the derived average StO2 level of 60% may correspond to StO2 values as low as 30% due to the variation of the derivation itself, or even lower than 30%.

![Figure 2 System calibration with different blood concentration and oxygenation](image)

**2.2 Animal model and imaging protocol**

The animal protocols involving studies on canine and rodent species were approved by the Institutional Animal Care and Use Committee of Oklahoma State University. The canine protocol was also approved after an on-site inspection by the U.S. Army Medical Research and Material Command. For this study a 7 year old, 27-kg sexually intact mixed-bred hound was used. The TVT cell line was propagated in non-obese-diabetic/severe combined-immunodeficiency (NOD/SCID) mice. The neoplastic TVT cells were recovered and homogenized for injection into the canine prostate gland parenchyma. Approximately a 2 cc suspension of $2.5 \times 10^6$ cells/mL of homogenized TVT cells were aseptically injected trans-perineally into the right lobe of the prostate using a 6-in. 16-gauge hypodermic needle via TRUS visualization (Fig. 3-a). The TVT cells were confined within the cranial aspect of right prostatic lobe during injection. The dog underwent weekly monitoring, including physical rectal examination, TRUS, Doppler US and trans-rectal NIR tomography, for 9 weeks and was then humanly euthanized for necropsy and histological examinations.
3. RESULTS AND DISCUSSIONS

3.1 Development of a transmissible venereal tumor visualized on NIR and TRUS

Shown in Figure 4 are the imaging results, over three weeks, of the right lobe around the site of tumor cell injection. In each column there are Doppler US, TRUS, [HbT] and StO2 images from the top row to bottom row. The optical images of [HbT] and StO2 were set to the same color-bar respectively for comparison. At baseline imaging, there was a hypo-echoic region in TRUS that was suspected as the pelvic lymph node that had a higher [HbT] and a lower StO2 than the prostate. At 7 days post injection, there was an indication of increased blood flow in the periphery of the injection site in the prostate which was consistent in both Doppler US and [HbT]. The region around the infused blood vessel had a higher StO2 than the adjacent prostate region. There was a region of higher [HbT] inside the prostate which was clearly visualized in optical images while unremarkable in TRUS. This region of higher [HbT] became a distinct hypo-echoic region in TRUS image at and after 14 days post injection.
At 8 weeks post-injection (Fig. 5), the hypo-echoic region in TRUS images increased in size as a result of the tumor growth. The Doppler ultrasound showed that the blood flow surrounded the tumor, which in optical imaging was presented as a region of high contrast of [HbT] encircling a region of low [HbT], while the StO2 of the region inside the indicated tumor site was low. This pattern of [HbT] and [StO2] indicated that the core of the solid TVT might have become necrotic.

3.2 A contra-lateral native cystic lesion visualized on NIR and TRUS
Figure 6 presents the images of the left lobe of the prostate which was visualized with a cystic region before the inoculation of the TVT cells. Shown in Figure 7 were the images of the cystic lesion taken at the 6th week post-injection. The cyst had consistently shown a moderate [HbT] contrast and significant lower StO2 compared with peripheral normal-appearing prostatic tissue. The region of low StO2 region was slightly greater than the region of higher [HbT] and definitively greater than the region of remarkably hypo-echoic in US.
3.3 Longitudinal changes of hemoglobin and oxygen saturation in regions of tumor and cyst

Average values of [HbT] and StO2 at regions corresponding to the growth of tumor mass and peripheral to the cyst (a circle with 1cm diameter that is approximately 200% of the area of the cyst shown on US) were calculated and compared in Fig. 8. At each weekly measurement, the reconstructed data were evaluated among three longitudinal locations from cranial, middle to caudal sites accordingly to minimize sensitivity issues and artifacts [20]. At the baseline measurement performed over a two week duration before the tumor cell injection, the [HbT] (blue line) indicating the normal prostate tissue had a little higher [HbT] than that indicating the cystic lesion, and also had higher StO2 than that indicating the cystic lesion. After injection of the tumor cells, the [HbT] at both lobes were increased which could have been due to the inflammational response of the prostate.
Around the cystic lesion, the [HbT] increased during the first two weeks after the injection and dropped back to the previous normal level after 4 weeks. In the right lobe at the site of the TVT cell injection, the [HbT] remained high for the following 5 weeks. During week 6 and week 7 the tumor site [HbT] decreased to a value similar to that of the normal tissue. After week 7 the blood supply around the tumor became significantly increased, which was shown as an increased [HbT] in week 8 and week 9 post-injection, while the StO2 decreased during the last four weeks.

3.4 Histological results

The dog was euthanized at 9 weeks post-inoculation of TVT and subjected to thorough gross and histological examination. Metastasis to the regional lymph nodes in the pelvic canal was found. The prostate was excised, and sliced sagittally by free-hand technique at positions approximately the same as those used for in vivo NIR and TRUS monitoring, specifically middle-line, half-way between middle-line and right edge, slightly medial to the right edge, half-way between middle-line and left-edge, and slightly medial to the left edge.

![Figure 9 Gross histology and H&E staining results.](image_url)

A cystic lesion was found in the middle of the left lobe that correlated with a cystic lesion seen during imaging (Fig. 9-a). The right lobe contained primarily TVT tumor masses at dorsal and
slightly cranial regions (Fig.9-b). Histologically, necrotic foci were observed in the solid tumor masses, which supported the minor results of low [HbT] and low [StO2] in the tumor-indicative region in Fig.5. Shown in Fig. 9-c is the H&E image of tissue excised from the periphery of the cystic lesion in Fig. 9-a, wherein a concentrated infiltrate of hemosiderophages (framed by arrows) indicates previous hemorrhage. The necrotic foci of the TVT mass shown in Fig. 9-b were confirmed by H&E staining as illustrated in Fig. 9-d.

SUMMARY

In summary, this work reported the in-vivo optical imaging of both [HbT] and StO2 changes associated with the growth of a TVT tumor in canine prostate during a 9-week time-source, and that associated with a native prostate cystic lesion during the same period of evaluation. This study demonstrated the potential to improve the accuracy of prostate cancer detection by applying multi-wavelength optical spectral measurement to augment the US and Doppler US imaging techniques.

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REFERENCES


