Comparison of tumor vascular blood volume measured by near infrared spectroscopy and ^{19}F NMR spectroscopy

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Near-infrared spectroscopy (NIRS) was used to measure changes of total hemoglobin concentration, $\Delta[\mathrm{Hb}]_{\mathrm{total}}$, and relative vascular oxygenation $\Delta[\mathrm{HbO_2}]$ in rat mammary and prostate tumors in response to hyperoxic gas interventions. ^{19}F NMR of vascular perflubron was used to compare with the NIRS observations. The consistent trends between $\Delta[\mathrm{Hb}]_{\mathrm{total}}$ and $\Delta V_{\mathrm{T-blood}}$ demonstrated that the NIRS can serve as an accurate, non-invasive, real time, monitoring tool for tumor vascular volume measurement. Meanwhile, these results also demonstrated that different types of tumors may respond to hyperoxic gases differently, and that NIRS could be used to assess vascular oxygenation changes non-invasively for guiding tumor therapy.

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Hemodynamic parameters, such as blood volume, blood oxygenation, and total hemoglobinconcentration ($[\mathrm{Hb}]_{\mathrm{total}}$), etc, possess essential information for the management of the critical illness. Effective monitoring of these parameters permit analysis of key circulatory functions and the anticipation of deterioration so that proactive treatments can be initiated[1,2]. Accordingly, accurate assessment of tumor vascular oxygenation and Hb concentration at various stages of tumor growth and in response to interventions may provide a better understanding of tumor development and may serve as a prognostic indicator for treatment outcome[3,4].

Near infrared spectroscopy (NIRS), with good temporal resolution and lower cost, provides such a noninvasive, non-ionizing, real time means to monitor the total hemoglobin concentration and oxygen saturation of tissue vasculature. Previously, we have demonstrated the practicability and accuracy of NIRS for the measurement of tumor vascular oxygenation changes ($\mathrm{HbO_2}$), under various therapeutic interventions, by comparative measurements with a pulse oximeter[5], needle electrode, MRI[6], and multi-channel FOXY$^{\mathrm{TM}}$ oxygen sensor[7]. In this study, we have conducted MRS measurement to validate the NIRS as an accurate means to be able to quantify changes in tumor vascular blood.

A homodyne, frequency-domain, NIRS system (NIM, Philadelphia, PA) used in this study has been described in detail previously based on modified Beer-Lambert's law[8], the changes in oxygenated, deoxygenated, and total hemoglobin concentrations, i.e., $\Delta[\mathrm{HbO_2}]$, $\Delta[\mathrm{Hb}]$, and $\Delta[\mathrm{Hb}]_{\mathrm{total}}$, respectively, because respiratory intervention are calculated using the following equations, which have been derived previously[9] as

$$\Delta[\mathrm{HbO_2}] = \frac{-2.658 \times \Delta\mathrm{OD}\ (758\ \mathrm{nm}) + 3.743 \times \Delta\mathrm{OD}\ (785\ \mathrm{nm})}{d \times \mathrm{DPF}}, \quad (1)$$

$$\Delta[\mathrm{Hb}] = \frac{2.238 \times \Delta\mathrm{OD}\ (758\ \mathrm{nm}) - 1.683 \times \Delta\mathrm{OD}\ (785\ \mathrm{nm})}{d \times \mathrm{DPF}}, \quad (2)$$

$$\Delta[\mathrm{Hb}]_{\mathrm{total}} = \Delta[\mathrm{HbO_2}] + \Delta[\mathrm{Hb}] = \frac{-0.42 \times \mathrm{OD}\ (758\ \mathrm{nm}) + 2.06 \times \mathrm{OD}\ (785\ \mathrm{nm})}{d \times \mathrm{DPF}}, \quad (3)$$

where $\Delta\mathrm{OD}(\lambda)$ represents a change in optical density at the specific wavelength, λ. $\Delta\mathrm{OD}(\lambda) = \log\ (A_{\mathrm{B}}/A_{\mathrm{T}}) = \varepsilon_{\mathrm{Hb}}(\lambda)\Delta[\mathrm{Hb}] + \varepsilon_{\mathrm{HbO2}}(\lambda)\Delta[\mathrm{HbO_2}]$. A_{B} and A_{T} correspond to light intensities measured under the baseline and transient conditions. $\varepsilon_{\mathrm{Hb}}(\lambda)$ and $\varepsilon_{\mathrm{HbO_2}}(\lambda)$ are extinction coefficients at wavelength λ for molar concentrations of deoxygenated hemoglobin and oxygenated hemoglobin, respectively. d is the source-detector separation, and the unit for $\Delta[\mathrm{HbO_2}]$, $\Delta[\mathrm{Hb}]$ is mM/DPF, where DPF is the differential path-length factor for tumor tissues. The experimental setup for NIR measurement is shown in Fig. 1.

For MRS measurement, an Omega CSI 4.7-Telsa, superconducting magnet system (Acustar$^{\mathrm{TM}}$, Bruker Instrument, Inc., Fremont, CA) was used for the measurement of tumor blood volume. The artificial blood substitutes Perfluorooctylbromide (PFOB) ($\mathrm{C_8F_{17}Br}$) emulsions (Alliance pharmaceutical Corp., San Diego, CA) was intravenously infused into the rat blood stream as a blood volume indicator for ^{19}F magnetic resonance spectroscopy (MRS) measurements. Either breast or prostate tumors were placed within a frequency-tunable (^{1}H/^{19}F),

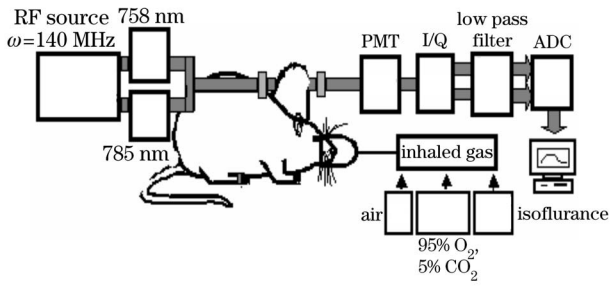

Fig. 1. Schematic diagram of experimental setup for the NIRS system.

single-turn, solenoid coil, accompanying with a sealed capillary containing sodium trifluoroacetate (TFA) used as an external standard for quantifying tumor blood volume. The rats were exposed to hyperoxic respiratory. Figures 2 shows the molecular structure and a typical ^{19}F MRS of PFOB emulsion.

Given a set of data acquisition parameters, the integration of ^{19}F signal from a tumor was linearly proportional to the total number of ^{19}F nuclear spins of PFOB in the tumor, which, in turn, was linearly proportional to the total blood volume in the tumor. After the tumor measurement, rats were removed from the RF coil, with the reference TFA capillary still left in the original position. Amount of 0.5-mL blood sample was then drawn by tail vein from the rats and was placed into the RF coil without disturbing the reference TFA capillary. The blood volume in tumor vasculature can be calculated based on

$$V_{\mathrm{T\_blood}} = V_{\mathrm{S\_blood}} \cdot \left(\frac{I_{\mathrm{T\_blood}}}{I_{\mathrm{S\_blood}}}\right) \cdot \left(\frac{I_{\mathrm{S\_TFA}}}{I_{\mathrm{T\_TFA}}}\right), \qquad (4)$$

where $V_{\mathrm{T\_blood}}$ and $V_{\mathrm{S\_blood}}$ were the tumor vascular blood volume and blood sample volume (mL), respectively, and $I_{\mathrm{T\_blood}}$ and $I_{\mathrm{S\_blood}}$ were the integrated NMR signals from the ^{19}F peaks of PFOB in the rat tumor and in the blood sample, respectively. $I_{\mathrm{blood\_TFA}}$ and $I_{\mathrm{tumor\_TFA}}$ were the respective integrations of ^{19}F NMR signals from the TFA capillary, which was used as a calibration standard to accompany with the blood sample and the tumor measurement.

Mammary adenocarcinomas 13762NF and Dunning prostate asenocarcinomas R3327-AT1 were implanted in skin pedicles on the forebacks of female Fisher 344 rats (∼150 g) and adult male Copenhagen rats (∼250 g), respectively. Once the tumors reached 1—2-cm diameter, tumors were shaved to improve optical contact for NIR light transmission.

Figure 3(a) shows the time course profiles of $\Delta[\mathrm{Hb}]_{\mathrm{total}}$ and $\Delta[\mathrm{HbO_2}]$ monitored by NIRS in response to hyperoxic gas interventions, i.e., air-carbogen-air-oxygen-air, for a representative breast tumor (2.6 cm^3). When the inhaled gas was switched from air to carbogen, $\Delta[\mathrm{Hb}]_{\mathrm{total}}$ increased significantly (p <0.0001) from baseline 0.002±0.006 to the maximum of 0.079±0.004 (mM/DPF) over the period of carbogen intervention. After the gas was switched back to air, a significant drop ($p < 0.0001$) of $\Delta[\mathrm{Hb}]_{\mathrm{total}}$ occurred, and followed by a plateau

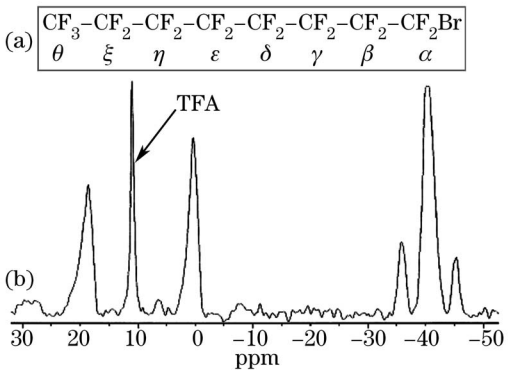

Fig. 2. Molecular structure of PFOB (a) and 19F NMR spectrum of PFOB emulsion in 2.5-mL Fisher rat blood, with TFA as an external label (b).

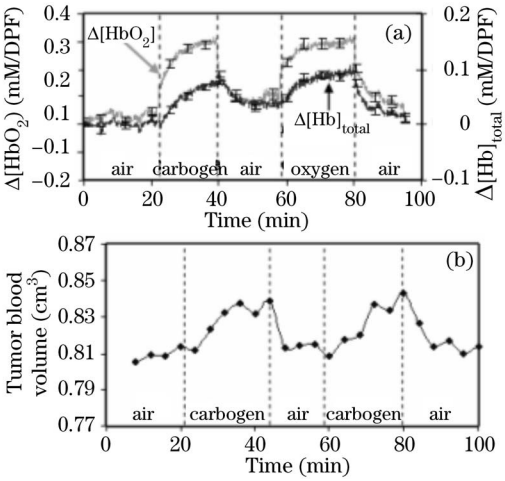

Fig. 3. (a) Time course profiles of tumor vascular D$[\mathrm{Hb}]_{\mathrm{total}}$ and D$[\mathrm{HbO_2}]$ monitored by NIRS for a representative breast tumor (2.6 cm^3), with an inhaled gas sequence of air-carbogen-air-oxygen-air. (b) Time course of tumor blood volume, $V_{\mathrm{T\_blood}}$, measured by ^{19}F MRS of PFOB for the same breast tumor (2.6 cm^3), with the gas breathing sequence same as that used in (a).

at 0.036±0.004 (mM/DPF). A similar temporal response pattern was repeated in the oxygen intervention cycle. The maximal magnitude change in $[\mathrm{Hb}]_{\mathrm{total}}$ due to oxygen intervention is about 0.067±0.004 (mM/DPF). Compared with $\Delta[\mathrm{Hb}]_{\mathrm{total}}$, $\Delta[\mathrm{HbO_2}]$ had a similar temporal profile with larger magnitude increases[5−7].

For comparison, the same rat was experienced in the same gas intervention sequence, i.e., air-carbogen-air-oxygen-air, during the ^{19}F MRS measurement in the next day. Figure 3(b) shows the tumor blood volume, $V_{\mathrm{T\_blood}}$, with respect to the gas interventions measured by ^{19}F MRS of PFOB for the same breast tumor (2.6 cm^3). This figure shows that $V_{\mathrm{T\_blood}}$ increased significantly (p <0.0001) from baseline 0.809±0.004 cm^3 to the maximum of about 0.837±0.004 cm^3 during carbogen intervention, followed by a quick return as air breathing was switched on. A similar profile was continued in the oxygen intervention cycle. Further comparison between Figs. 3(a) and 3(b) exhibits the overall consistent trend between $V_{\mathrm{T\_blood}}$ and $\Delta[\mathrm{Hb}]_{\mathrm{total}}$.

The results obtained from prostate tumors were often different from those taken from the breast tumors (Fig. 4). Carbogen interventions in both of the cycles did not produce significant changes in $\Delta[\mathrm{Hb}]_{\mathrm{total}}$, but $\Delta[\mathrm{HbO_2}]$ showed good response in the second carbogen intervention. To verify $\Delta[\mathrm{Hb}]_{\mathrm{total}}$ obtained from NIRS, the same rat was performed with the MRS experiment for blood volume measurement in the following day. Data in Fig. 4 shows that little change in tumor blood volume was observed during the carbogen interventions, being consistent with the result taken from NIRS for $\Delta[\mathrm{Hb}]_{\mathrm{total}}$.

In this study, the feasibility of NIRS for the measurements of $\Delta[\mathrm{Hb}]_{\mathrm{total}}$ was evaluated by comparing with the blood volume measurement using ^{19}F MRS. These experimental results demonstrate that tumor vascular $\Delta[\mathrm{Hb}]_{\mathrm{total}}$ determined by NIRS and tumor blood volume $V_{\mathrm{T\_blood}}$ obtained from ^{19}F MRS is consistent one another under hyperoxic gas interventions. Since ^{19}F MRS

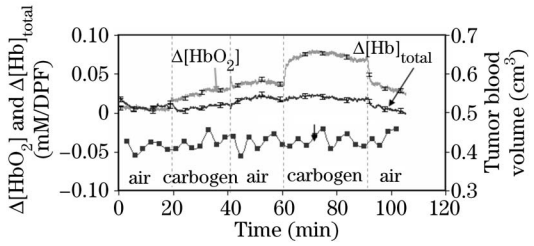

Fig. 4. Time course profiles of tumor vascular $D[Hb]_{total}$, $D[HbO_2]$ monitored by NIRS and tumor blood volume measured by ^{19}F NMR of perflubron for a representative prostate tumor (8.9 cm^3), with respect to repeated carbogen interventions.

of PFOB is considered as a well-established method for blood volume measurement, the comparative study between the NIRS and ^{19}F MRS presented in this paper validates the correctness and reliability of NIRS for determination of tumor vascular total hemoglobin concentration. Furthermore, while ^{19}F MRS of PFOB can be applied to deep tumors with special pulse sequences, NIRS can be used to monitor the acute response of tumors to different therapeutic interventions. The longitudinal measurements of tumor blood volume by MRS and hemoglobin concentration by NIRS could reveal insight into tumor development and perhaps provide a prognostic indicator for tumor therapy. In another hand, the simultaneous measurements in $\Delta[Hb]_{total}$ and tumor blood volume provide an experimental modality to quantify the fraction of tumor blood sampled by NIR system[12].

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