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Abstract. Despite the widespread use of radio frequency (RF) ablation, an effective way to assess thermal tissue damage during and after the procedure is still lacking. We present a method for monitoring RF ablation efficacy based on thermally induced methemoglobin as a marker for full tissue ablation. Diffuse reflectance (DR) spectra were measured from human blood samples during gradual heating of the samples from 37 to 60, 70, and 85°C. Additionally, reflectance spectra were recorded real-time during RF ablation of human liver tissue *ex vivo* and *in vivo*. Specific spectral characteristics of methemoglobin were extracted from the spectral slopes using a custom optical ablation ratio. Thermal coagulation of blood caused significant changes in the spectral slopes, which is thought to be caused by the formation of methemoglobin. The time course of these changes was clearly dependent on the heating temperature. RF ablation of liver tissue essentially led to similar spectral alterations. *In vivo* DR measurements confirmed that the method could be used to assess the degree of thermal damage during RF ablation and long after the tissue cooled. [DOI: 10.1117/1.JBO.19.9.097004]

Keywords: radio frequency ablation; reflectance spectroscopy; treatment monitoring; liver.

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1 Introduction

Radio frequency (RF) tumor ablation is a thermal ablation technique using a needle-type electrode that is inserted into malignant tissue. The technique is widely used for the treatment of malignant lesions in the liver, kidneys, and lungs. To completely destroy a tumor, the entire lesion must be heated to cytotoxic temperatures. However, lesion size and local blood flow often complicate heating of the entire tumor volume, resulting in heterogeneity of heat deposition throughout a given lesion to be treated. Determining whether a complete tumor ablation has been achieved is difficult as there is no method to accurately evaluate the extent of the ablation zone. The local tumor recurrence rates after liver RF ablation vary significantly between published series, with local tumor recurrence rates of 3.6% (Refs. 1 and 2) to 60%,[3] where the latter is mainly due to incomplete ablation of the tumor margin. Real-time monitoring could contribute to locally effective destruction of tumor tissue in combination with a preservation of healthy liver tissue.

Optical spectroscopy techniques, such as diffuse reflectance (DR) spectroscopy at the tip of a thin fiber-optic needle, may enable real-time monitoring by measuring specific physiological information from the examined tissue. Unlike temperature monitoring with use of thermocouples, which measure the temporary effects of ablation, DR spectroscopy could be used to detect persistent chemical and structural changes undergone by tissue during thermal ablation. This may allow real-time monitoring of the progress of ablation and the adequacy well after the ablation has been completed.

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Various groups have successfully focused on spectroscopic detection of thermal damage of liver tissue. It was shown that an increase in reflectance intensity and a decrease in overall fluorescence intensity correlated with the histological degree of thermal damage.[4–6] However, focusing on absolute spectral intensities as an endpoint is prone to be affected by needle movement, pooling of (coagulated) blood around the probe tip, and differences in instrumentation or calibration. Instead of evaluating the absolute magnitude of the spectrum, we propose to use semiquantitative information extracted from the spectra that indicates irreversible tissue injury. Such a method may be more sensitive to subtle heat-induced changes and may provide advanced characterization of irreversible tissue damage.

Depending on the duration of heating and the tissue susceptibility for thermal damage, irreversible tissue damage occurs at a threshold temperature of ~60°C.[7] It is, therefore, important to identify markers that indicate whether or not this threshold temperature has been reached. When tissue is subjected to increasing heat, tissue proteins start a denaturation process and undergo irreversible structural changes. In addition, methemoglobin (metHb) is formed from hemoglobin at temperatures >60°C, making it a potential marker for irreversible liver tissue damage.[8]

The group of Tromberg developed a broadband diffuse optical spectroscopy (600 to 1000 nm) method to derive tissue concentrations of metHb and four other chromophores. Chromophore concentrations could accurately be monitored *in vivo*, despite significant overlapping spectral features.[9] Formation of metHb following heat exposure has been reported by a few other studies for a variety of clinical applications. Barton et al. found that changes in the optical properties of

hemoglobin in a skin model do occur during laser irradiation with a 532 nm wavelength based on the detection of thermally induced metHb.[10] More recently, Randeberg et al. showed that measurements of the average metHb concentrations in port-wine stains and telangiectasia veins immediately after laser exposure may be used to verify that the blood temperature has been sufficiently high to induce thermal damage to the vessel wall.[11] In the same way, high amounts of spectroscopically detected metHb appeared to be a good indicator of nonviability of thermal wounds.[12]

The aim of the present study is to investigate whether DR spectroscopy could be used to assess the efficacy of RF tumor ablation.

2 Materials and Methods

2.1 *Spectroscopic System*

Reflectance spectra were acquired using a portable spectroscopic system as illustrated in Fig. 1. For illumination of the tissue, a white light halogen broadband light source (360 to 2500 nm) with an internal shutter was used. The tissue was probed using a clinical-grade disposable 16 G fiber-embedded needle (INVIVO, Schwerin, Germany). The probe had one fiber (200 μm) connected to the light source and another fiber (200 μm) connected to a spectrometer (silicon detector; Andor Technology, Belfast, United Kingdom, DU420A-BRDD), optimized for wavelengths between 400 and 1050 nm with a spectral resolution of ~4 nm. The center-to-center distance between the emitting and collecting fibers was 0.34 mm. The probe had a polished angle tip of 72 deg to minimize tissue damage during insertion, while the fiber ends were cut straight. The spectroscopy needle was made from materials that are heat-resistant in the temperature range that was investigated. DR spectra were acquired with a 0.3 to 1.0 s integration time, depending on the signal intensity at the start of each experiment. The integration time was kept constant during each experiment. The system was controlled by a custom-made LabView software user interface (National Instruments, Austin, Texas, USA).

2.2 *Preparation and Heating of Blood Samples*

Human venous blood (hematocrit = 41%) was obtained from a healthy human donor. The blood was preserved in an ethylenediaminetetraacetic acid (EDTA) tube to prevent coagulation. In the visible wavelength range, the optical absorption of hemoglobin in blood is dominant compared to the scattering due to red blood cells. To enhance diffuse reflectance of the samples, Intralipid®-20% was added as a highly scattering medium. Saline was added to obtain a blood concentration representative for liver tissue.[13] The stock solution that was prepared contained 10% blood, 70% saline, and 20% Intralipid®-20%.

To evaluate the effect of thermal coagulation, six samples of the stock solution were placed in 1.5 ml cuvettes and heated from 37 to 60, 70, and 80°C, respectively. For this purpose, a thermostat-controlled dry block heater (Techne, Staffordshire, United Kingdom DB-2D) was used. Once the set temperature was reached (after 3 to 5 min), the temperature was maintained for 15 min. The temperature of the samples was monitored by placing a digital thermometer in a dummy sample. Reflectance spectra of the samples were acquired continuously with an interval of ~60 s during the whole procedure.

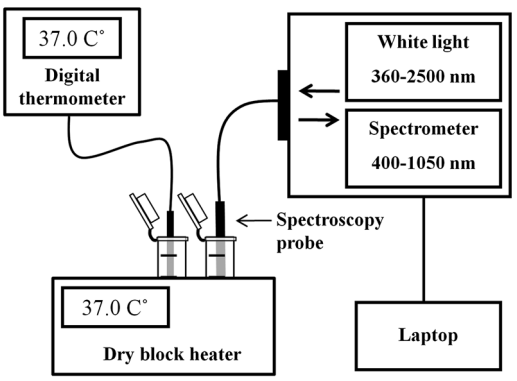

Fig. 1 Schematic overview of the spectroscopy setup.

2.3 *Ex Vivo RF Ablation Monitoring*

Prior to any human tissue experiments, it was confirmed that the obtained temperatures did not affect the spectral acquisitions in any way. This was done by heating Intralipid®-20% to the typical maximum temperatures achieved during RF ablation [60 to 90°C (Ref. 14)].

Ex vivo reflectance spectra were acquired during RF ablation on two human liver resection specimens after partial hepatic resection for colorectal liver metastases. Within 10 min. after partial hepatic resection, the freshly excised tissue was grossly inspected by the surgeon and released for the experimental procedure. Under ultrasound (US) guidance, an RF electrode (Cooltip™ RF ablation system) was placed in the tumor such that an ablation zone of 4 cm could be achieved, including surrounding normal liver parenchyma. Using US imaging, the spectroscopy needle was inserted into the liver parenchyma directly outside the tumor, but within the expected zone of ablation. Reflectance spectra were continuously acquired (interval ~30 s) during the whole ablation procedure and continued for 5 min after the ablation was terminated in order to investigate any reversible spectroscopic changes. US was used to confirm that the spectroscopy needle tip was located within the coagulated tissue.

2.4 *In Vivo Study Procedures*

To investigate heat-induced spectral changes *in vivo*, reflectance measurements were performed during an open RF procedure (4 to 8 min) during a laparotomy in a patient with unresectable colorectal liver metastases. This study was performed at The Netherlands Cancer Institute under approval from the internal review board committee (Dutch Trial Register NTR2557).

Just as in the *ex vivo* experiments, intraoperative US imaging was used for accurate positioning of the RF electrode and the spectroscopy needle. The RF electrode was inserted in the tumor, whereas the spectroscopy needle was placed just outside the tumor through a standard 14G guidance cannula (INVIVO) and was not further manipulated. Two sets of 20 reflectance spectra were acquired from exactly the same location before and after ablation. After performing the optical measurements, the spectroscopy needle was retracted and a 16 G core needle biopsy from the measurement site was taken through the same cannula. The sample was stored on-site at −80°C to allow for histopathological evaluation. Ablation was performed under standard operating procedures (according to the manufacturer's

guidelines) over a 12-min period, using an internally cooled tripod RF electrode (Cooltip™ RF ablation system).

2.5 *Histological Evaluations and Thermal Damage Assessment*

At the pathology department, the tissue samples were processed using a marker for cell metabolism nicotinamide adenine dinucleotide (NADH) diaphorase to determine the degree of cell death. All slides were reviewed by a single pathologist blinded to the tissue treatment. Viable tissue (positive staining) was defined by a blue color on NADH staining. Nonviable tissue remained unstained (negative staining) and was typically pink or yellow.

2.6 *Spectral Data Processing and Derivative Analysis*

The measured reflectance spectra were preprocessed using a Butterworth second-order low-pass filter to reduce signal noise. To investigate subtle changes in the shape of the reflectance spectra, a first-order derivative analysis was applied. The following equation was used to calculate the spectral slope (first-order derivative) from the filtered spectra:

$$R'(\lambda_i) = \frac{R(\lambda_i + 1) - R(\lambda_i)}{\lambda_{+1} - \lambda_i}, \quad (1)$$

where λ_{i+1} and λ_i are the adjacent wavelengths. $R(\lambda_i)$ and $R'(\lambda_i)$ are the original reflectance measurement and corresponding spectral slope at band λ_i, respectively. The reflectance measurements and corresponding spectral slope were evaluated for wavelengths ranging from 450 to 800 nm.

Previous research has shown that hemoglobin derivates, such as oxyHb and deoxyHb and bile are important chromophores in the liver because they significantly absorb in the visible wavelength range.[15–17] Figure 2 shows the absorbance spectra for oxyHb,[15] deoxyHb,[15] metHb,[15] and bile,[18] and the corresponding spectral slopes over the range of 450 to 800 nm. DeoxyHb has an absorption maxima at 556 and 758 nm, whereas oxyHb shows twin peaks at 546 and 577 nm. When inspecting the reflectance measurements of the RF ablated liver, it was observed that an additional absorption feature was present between 600 and 650 nm. In the literature, it has been shown that a chemical change occurs in hemoglobin at a critical temperature creating metHb, an oxidized form of hemoglobin, which is incapable of exchanging oxygen.[10] MetHb absorbs at 633 nm, with a positive peak that is not affected by other components [Fig. 2(a)]. This absorption feature sharply declines between 633 and 700 nm and results in a characteristic peak in the spectral slope at ~645 nm [Fig. 2(b)].

Examples of a reflectance measurement (R) of fully coagulated blood (85°C; >10 min) and the corresponding spectral slope (R') are shown in Fig. 3. The reflectance spectrum measured prior to heating has a clear oxyHb signature, as shown in Fig. 3(a) (blue line). The reflectance measured after full coagulation of the sample was spectrophotometrically identified as metHb. There is a pronounced increase in absorption between 600 and 700 nm. The latter results in a small negative peak around 630 nm and a strong positive peak at 675 nm in the spectral slope, as shown in Fig. 3(b). Since the wavelength of the peak minima and maxima may shift due to changes in light scattering, automatic peak detection was used to determine

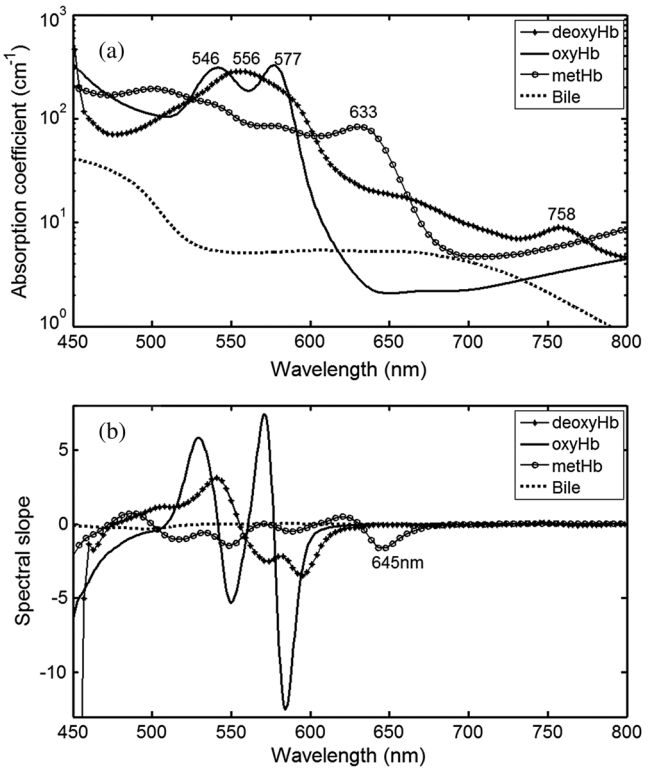

Fig. 2 Extinction coefficients (a) and corresponding spectral slope (b) for oxyHb, deoxyHb, bile, and metHb. Data from Refs. 15 and 18.

the exact wavelength of these peaks for each individual measurement. For all reflectance measurements, parameterization [denoted as Y; Fig. 3(c)] was achieved by calculating the difference between both peaks in the spectral slope using the equation

$$Y = R'(\lambda_2) - R'(\lambda_1), \quad (2)$$

where λ_1 and λ_2 indicate the positions of the metHb peaks in the spectral slope. An optical ablation ratio (OAR) was then calculated by using the absolute values of $R'(\lambda_1)$ and $R'(\lambda_2)$ to eliminate the effect of variation in amplitude of the original reflectance curves.

$$OAR = \frac{R'(\lambda_2) - R'(\lambda_1)}{|R'(\lambda_1)| + |R'(\lambda_2)|}. \quad (3)$$

3 Results

3.1 *In Vitro Heated Blood Samples*

Reflectance spectra obtained from the heated blood samples and corresponding spectral slopes are shown in Fig. 4. Within the wavelength range, the spectra of the nonheated samples (blue lines) were largely dominated by oxyHb (absorption at 546 and 577 nm). Thermal coagulation led to significant changes in the spectral shape. Heating at temperatures >60°C caused attenuation of oxyHb absorption features and a decrease in reflectance above 600 nm. This led the spectral slopes to a decrease of the oxyHb-related features (minima at 525 and 570 nm, maxima at 596 nm) and the occurrence of an addition peak around 650 to 675 nm. It should be noted that the

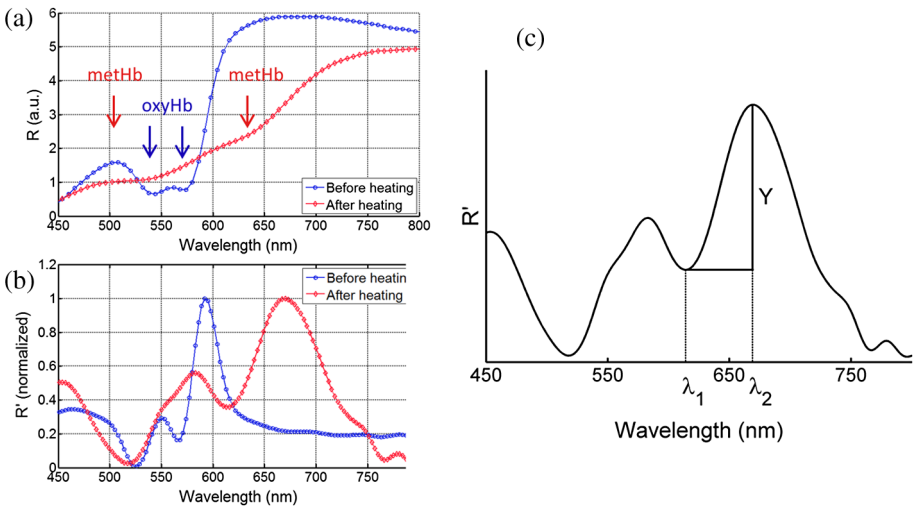

Fig. 3 Examples of reflectance spectra (R) of a human blood sample, measured before (blue lines) and after (red lines) full coagulation. Intensities are given in arbitrary units. In (b), the corresponding spectral slopes (R') are shown. The values of R' are normalized to the maximal intensity between 450 and 800 nm. (c) illustrates how peaks in the spectral slopes were used for quantification of metHb.

maximum in the spectral slopes that initially appeared around 650 nm was red-shifted throughout the heating process.

The dynamics of the observed spectral changes were evaluated by calculating the OAR over time for each sample. Results are shown in Fig. 5. The rate of the observed alterations was clearly dependent on the set temperature. At 60°C, a small increase in OAR was observed after 15 min of heating, whereas at temperatures of 70 and 80°C, a much larger change in OAR was seen within a few minutes of heating. The time course of the OAR could roughly be divided into three stages: a period of negligible change in the spectral shape, a steep increase in OAR, and a plateau phase. The third stage was not achieved for the sample heated at a temperature of 60°C for more than 15 min.

3.2 *RF Ablation Monitoring*

To investigate spectral changes occurring during RF ablation of liver tissue, reflectance measurements ($n = 23$ and $n = 29$ spectra) were continuously acquired from two human liver resection specimens during RF ablation.

Figure 6 shows a typical example of the time course of the reflectance spectra [Fig. 6(a)] and corresponding spectral slopes [Fig. 6(b)], during a representative RF ablation experiment *ex vivo*. The ablation was started at $t = 0$ min and finished after 7.5 min. To facilitate changes in spectral shape, the reflectance measurements [Fig. 6(a)] were normalized using the reflectance intensity value at 800 nm. The maximal change in the reflectance and spectral slopes was observed approximately

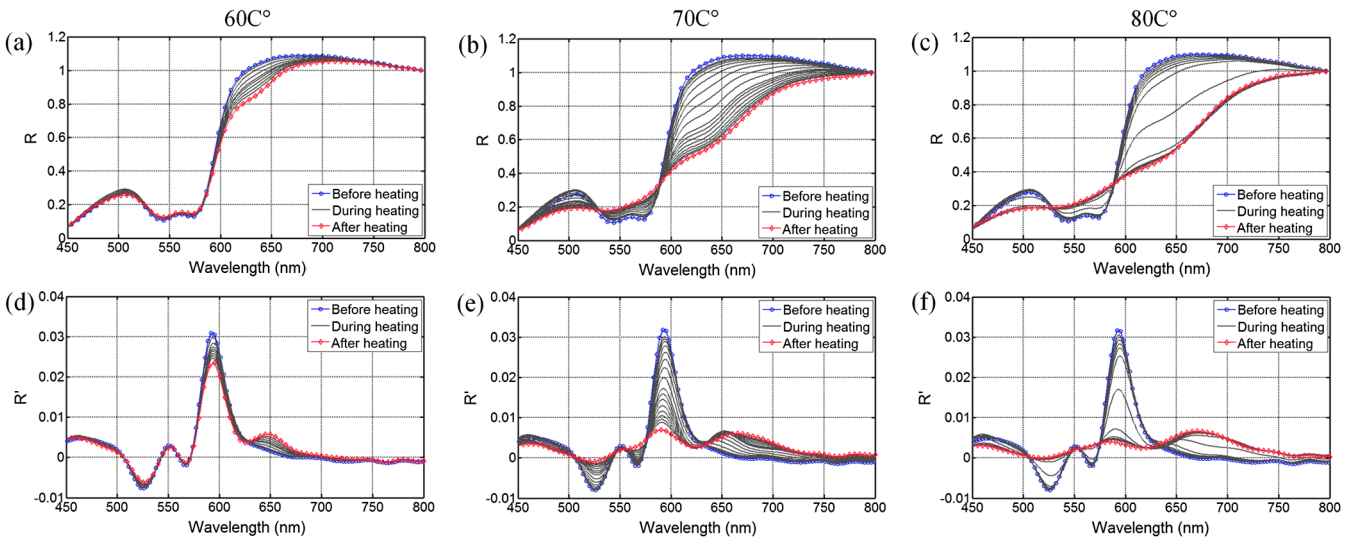

Fig. 4 Reflectance and corresponding spectral slopes obtained from blood samples heated to 60, 70, and 80°C. To facilitate comparison between samples, reflectance spectra were normalized using the reflectance intensity value at 800 nm. Note the increasing value for the positive peak at 650 to 675 nm in the spectral slopes.

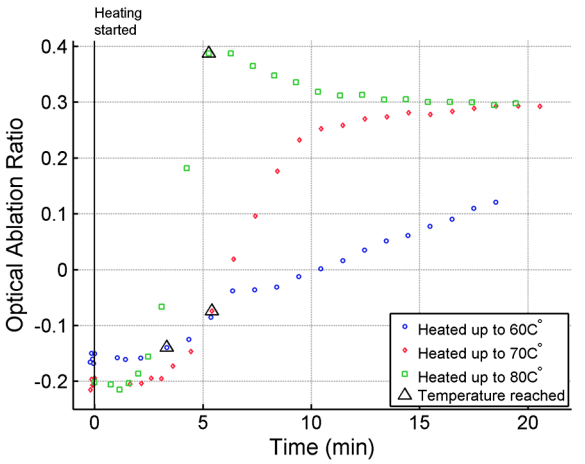

Fig. 5 Longitudinal change in optical ablation ratio for the blood samples heated to 60, 70, and 80°C.

2 min after the start of the ablation. No obvious changes in spectral shape occurred during the 5 min after the ablation was terminated, indicating that irreversible alterations were measured. The time course of the OAR during RF ablations [Fig. 6(c)] showed the same characteristic profile as that observed for the heated blood samples. The changes in OAR consistently corresponded with the observed changes in the spectral shape.

To validate the previous findings in a clinical setting, reflectance spectra were measured *in vivo* before and after a full ablation of a liver metastasis of colorectal origin. The initial (i.e., nonablated) and final (i.e., last recording) reflectance spectra, spectral slopes, and corresponding histopathology images are shown in Fig. 7. Spectral changes were comparable to the ones seen during the *ex vivo* ablation experiments. The OAR markedly increased from 0.13 to 0.51. Macroscopic evaluation and histological analysis confirmed that the spectra acquired after RF ablation were performed in fully ablated liver parenchyma.

4 Discussion

Despite the widespread use of RF ablation, an effective way to assess thermal tissue damage during and after the procedure is still lacking. To our knowledge, this report demonstrates the first published results of first-derivative DR spectroscopy to assess the efficacy of RF ablation *in vivo*.

In this study, reflectance measurements were performed during the heating of human blood samples to various temperatures and during RF ablation of human liver tissue both *ex vivo* and *in vivo*. Thermal coagulation of blood samples caused significant changes in the spectral shape, which is attributed to the thermal conversion of hemoglobin to metHb. RF ablation of liver tissue essentially led to similar spectral alterations. Specific spectral characteristics were extracted from the spectral slopes using an OAR. For the heated blood samples, the longitudinal changes in OAR were clearly dependent on the heating temperature and could be divided into three stages. In the first stage, minimal spectral change was observed. During the heating of the (oxygenated) blood samples, deoxyHb occurred as a transient intermediate of oxyHb. We hypothesize that this is due to hemoglobin's decreased affinity for oxygen with an increase in temperature, a so-called right shift in the oxygen-hemoglobin

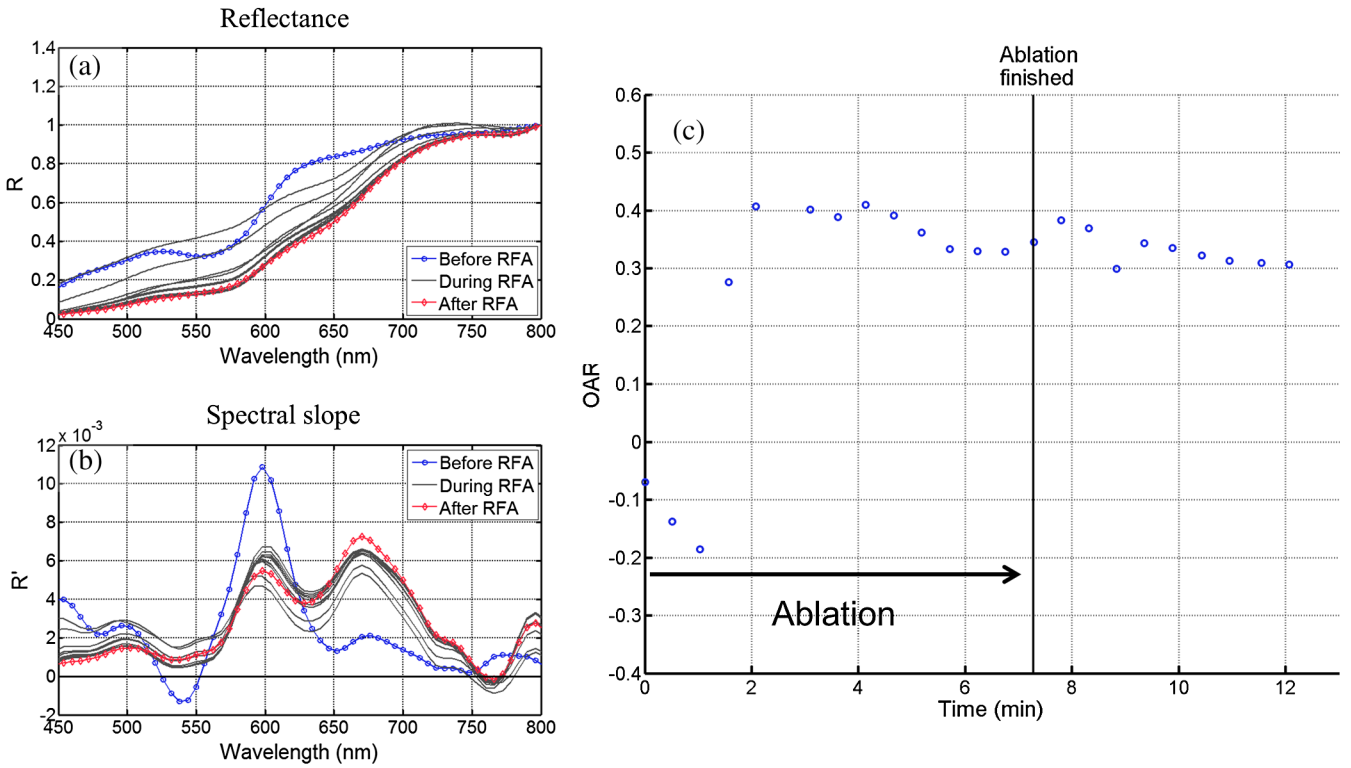

Fig. 6 Radio frequency (RF) ablation monitoring *ex vivo*. Heat-induced spectral changes in reflectance spectra (a) and spectral slopes (b) during an RF ablation of human liver tissue *ex vivo*. Only 12 out of 23 spectra are shown (sampled 1:2) to allow better visualization. (c) shows the time course of optical ablation ratio, as calculated from the spectral slopes.

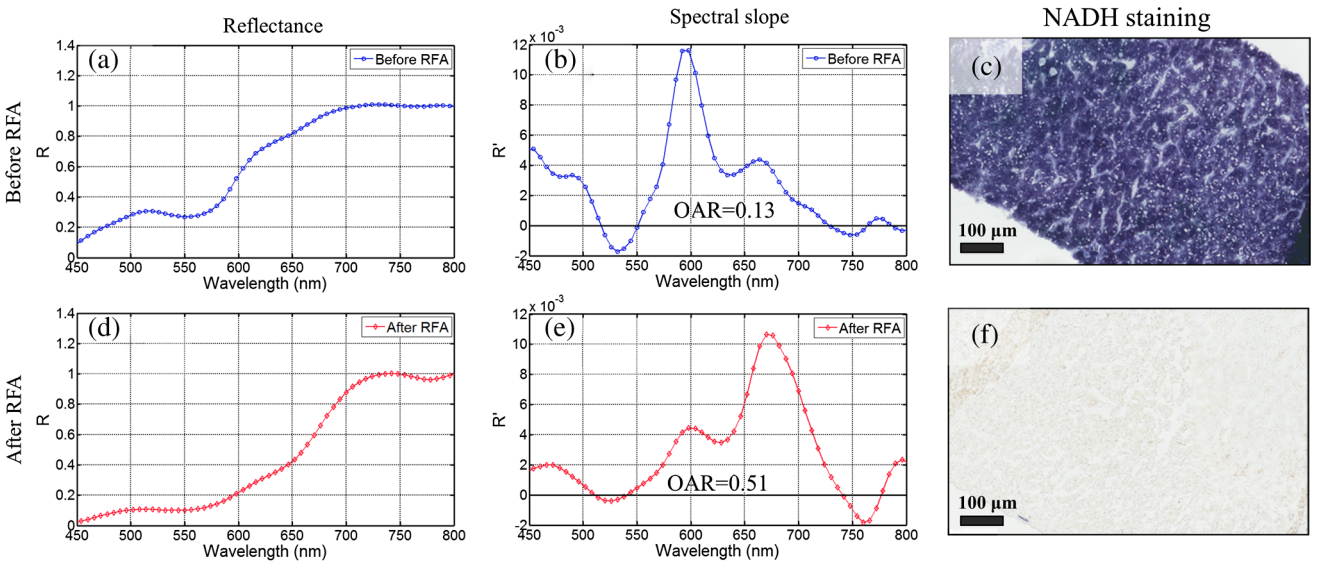

Fig. 7 Correlation of thermal tissue damage with spectral changes measured *in vivo* during open RF ablation. The spectra shown in (a) and (b) were measured in native liver parenchyma prior to ablation, whereas (d) and (e) show corresponding spectral acquisitions after RF ablation. The blue color in (c) indicates viable liver parenchyma, whereas nonviable tissue remained unstained (f).

dissociation curve.[19] When the threshold heat exposure that is needed for permanent alterations of hemoglobin had been reached, this resulted in a steep monotone increase in the OAR (defined as stage 2). In stage 3, a plateau was reached, at which point no further spectral alterations occurred.

To investigate the thermal tissue damage occurring during RF ablation, a series of ablations of human liver tissue was performed *ex vivo*. An important finding in our experiment was that changes in spectral characteristics achieved during ablation persisted after ablation was terminated and tissue was allowed to cool. As discussed, this is mainly attributed to thermally induced metHb formation. No significant chemical conversion of metHb is expected within a short period (hours) after cooling of the fully ablated tissue. Due to the coagulation of blood vessels, the ablated tissue has been isolated from perfusion and all enzymes are expected to be denatured.[20] In this way, our method would allow evaluation of the ablation margins well after the ablation has been completed.

The works of Ritz et al.[21] and others[10,11,22–26] provide a solid basis for understanding the changes in the optical properties of biological tissues under the effect of heating to increasing temperatures. Formation of metHb following heat exposure has been reported by several authors.[8,10,11,27,28] In addition, at temperatures >60°C, there is rapid tissue coagulation, as proteins denature and undergo irreversible structural changes.[7] The latter leads to an increase in the reduced scattering coefficient and associated increase in reflectance.[21,26] This is the reason why thermally coagulated tissue looks paler than normal tissue. This principle was exploited by Anderson et al.,[5] who performed RF ablation on healthy animals and monitored reflectance measurements through a fiber-optic probe. Empirical methods were used for analysis of reflectance spectra in which the spectral intensities at certain wavelengths were correlated with the degree of thermal damage during RF ablation. They found that an increase in the absolute reflectance intensity correlated with the histological degree of thermal damage. Similar results were achieved by Hsu et al., who performed spectral measurements on both animal and human liver tissue.[29] The time course of spectral changes observed in Anderson are consistent with the three characteristic stages of ablation observed in the present study.

In the study by Anderson, spectral changes occurred as the ablation zone progressed past the spectroscopy probe. During the first stage, spectra showed minimal deviation from the spectral shape of native liver parenchyma. Stage 2 changes occurred as the advancing hemorrhagic zone reached and passed the spectroscopic probe, whereas stage 3 changes occurred only when the liver tissue was fully coagulated. However, the absolute magnitude of a reflectance spectrum is dependent on a subtle variation in probe-to-tissue coupling and pressure, making it difficult to obtain reproducible and reliable reflectance spectra from the measured tissue.[30–32]

This study differs from previous studies in various ways. First, using derivative spectroscopy, we mainly focused on the spectral characteristics of metHb, while eliminating the magnitude difference and suppressing the background effects from scattering and other substances (e.g., oxyHb, deoxyHb, and bile). We showed that calculating the slope of the reflectance spectra can be applied to follow subtle changes in the shape of spectral bands. Second, by performing spectroscopy *in vivo* during open RF ablation, we demonstrated that the identified spectral characteristics could be used to assess the degree of thermal damage after RF ablation after the tissue temperature had normalized.

The feedback information provided by DR spectroscopy, as deployed in a fiber-optic needle, can help the interventionist in multiple ways. When the tumor being targeted is located near vital structures that might be damaged by heating (e.g., gall bladder, major blood vessels, bowel), a spectroscopy needle can be placed at a critical point away from the tumor. Real-time monitoring of tissue during an ablation procedure could then be used to determine when a particular level of tissue damage has been reached and, therefore, reduce the chance of local recurrence, while preserving surrounding healthy tissue. Furthermore, DR spectroscopy could be used directly after RF ablation to check focal areas of tissue that are suspected for

inadequate ablation. This may improve procedure outcome and disease-free survival. Further research is needed in which three-dimensional spectroscopic information is acquired at various distances from the ablation electrode during and after RF ablation.

Although the results of the present study are of specific interest for liver RF ablation, analogue results were observed in other fields, such as laser photocoagulation of vascular skin lesions[10,11] and assessment of skin burns.[12] Interestingly, similar results were observed in the area of cardiac ablation monitoring, where methmyoglobin was found in ablated cardiac muscle tissue, as described by Swartling et al.[33] These results make DR spectroscopy a promising diagnostic tool to verify irreversible thermal damage for heat-based therapy in general.

It should be noted that the results presented here are subject to some uncertainties. Although derivative analysis is insensitive to slow changes in the measured reflectance curve, alterations in scattering slope may have influenced the calculated values for the OAR to a certain extent. For example, in the spectral slope shown in Fig. 6(c), it can be observed that the metHb peak at ~660 nm shifted toward higher wavelengths. This is expected due to an increase in the scattering within the tissue. To minimize the effect on the exact value for the OAR, automated peak detection was used. Furthermore, during the heating of blood samples and tissue, the spectra of various tissue chromophores, including oxyHb and deoxyHb, may change shape and slightly shift to red wavelengths (bathochromic shift). The influence of these dynamic optical property changes on the OAR were not further studied here. Another uncertainty is the exact temperature and heating time needed to cause irreversible thermal damage.

From a clinical point of view, an ideal marker for RF ablation should provide a reliable, quantitative prediction as to whether or not tissue has been adequately ablated. The presence of such a marker should, therefore, be correlated with the extent of the thermal damage achieved. We have demonstrated that thermal coagulation of liver tissue can be quantified by using specific spectral information, which is expected to be due to rapid thermal conversion of hemoglobin to metHb. A strategy using a combination of the reflectance intensity, as mentioned earlier, and features extracted from the spectral shape may have the potential to improve the overall sensitivity of a future instrument.

Furthermore in humans additional studies to evaluate the exact relation between the proposed spectral markers and extend of thermal tissue damage are needed. This will provide a means to directly validate the quantitative physiological aspects of this technique in a clinical setting and may directly increase the clinical success rate of RF ablations of tumor lesions in liver, lung, and renal cancer.

5 Conclusion

In summary, this study shows the potential of real-time liver ablation monitoring by reflectance measurements at the tip of a needle. We have presented evidence that the thermal coagulation of liver tissue involves significant changes in the spectral slope, which is thought to be due to the thermal formation of metHb. This opens the potential to dynamically monitor the extent of irreversible thermal tissue damage based on these spectral features. Currently, a more extensive *in vivo* human study is being performed as a next step toward clinical implementation.

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