Real-time optoacoustic monitoring of temperature in tissues

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Abstract

To improve the safety and efficacy of thermal therapy, it is necessary to map tissue temperature in real time with submillimetre spatial resolution. Accurate temperature maps may provide the necessary control of the boundaries of the heated regions and minimize thermal damage to surrounding normal tissues. Current imaging modalities fail to monitor tissue temperature in real time with high resolution and accuracy. We investigated a non-invasive optoacoustic method for accurate, real-time monitoring of tissue temperature during thermotherapy. In this study, we induced temperature gradients in tissue and tissue-like samples and monitored the temperature distribution using the optoacoustic technique. The fundamental harmonic of a Q-switched Nd:YAG laser ($\lambda = 1064$ nm) was used for optoacoustic wave generation and probing of tissue temperature. The tissue temperature was also monitored with a multi-sensor temperature probe inserted in the samples. Good agreement between optoacoustically measured and actual tissue temperatures was obtained. The accuracy of temperature monitoring was better than 1°C , while the spatial resolution was about 1 mm. These data suggest that the optoacoustic technique has the potential to be used for non-invasive, real-time temperature monitoring during thermotherapy.

1. Introduction

Non-invasive real-time measurement of temperature distribution in tissues during thermotherapy is necessary for the safe and efficient thermal destruction of tumours or other abnormal tissues (Lehmann 1990, Welch and van Gemert 1995). Wide clinical application of thermotherapy is limited due to the lack of temperature control over the heated volume. Safe and efficient thermotherapy requires real-time temperature measurements with 1 mm spatial resolution and 1°C or better accuracy.

Infrared thermography is capable of real-time temperature measurements with an accuracy of 0.1°C . However, this

technique can measure temperature only from superficial tissue layers (Welch and van Gemert 1995). Ultrasound methods can provide real-time images with good resolution and depth of penetration, but they lack sufficient accuracy for temperature measurements (Seip and Ebbini 1995, MaassMoreno and Damianou 1996, Seip *et al* 1996). MRI requires a long acquisition time to provide temperature mapping with high resolution and accuracy (Graham *et al* 1998, Steiner *et al* 1998).

The laser optoacoustics (OA) technique has been developed and applied in many areas of biomedicine (Karabutov *et al* 2003, Wang 2003). For example, the OA method was successfully applied for breast cancer imaging (Esenaliev *et al* 1999, Kirillov *et al* 1999, Wang *et al* 2002, Andreev *et al* 2003, Copland *et al* 2004), functional and structural imaging of the brain (Wang *et al* 2003a, 2003b,

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Figure 1. Experimental set-up for optoacoustic measurements of temperature in tissue and tissue phantoms.

(This figure is in colour only in the electronic version)

2004), and real-time monitoring of oxygenation (Esenaliev *et al* 2002) and total haemoglobin concentration (Esenaliev *et al* 2004). Recently, we used the OA technique to monitor the cooling and freezing of tissues (Larin *et al* 2002); this can be applied for precise cryotherapy. All these applications utilize differences in optical properties between normal and modified tissue states.

That the OA technique is capable of non-invasive temperature determination of fungus of the eye during laser irradiation has recently been demonstrated in (Schule *et al* 2004). It has been shown that time-resolved detection and analysis of the laser-induced pressure profiles allow for reconstruction of OA images and calculation of tissue temperature with an accuracy of about 17%. In this paper, we study the capability of the OA technique to monitor the temperature distribution deeply in strongly scattering tissues and tissue phantoms, at temperatures typical for hyperthermia.

2. Materials and methods

2.1. Experimental set-up

The experimental set-up used in this study is shown in figure 1. The fundamental ($\lambda = 1064\text{ nm}$) harmonic of a Q-switched nanosecond Nd:YAG laser was used for optoacoustic wave generation. A diverging lens was used to provide a wide (10 mm in diameter) laser spot on the sample. The energy of each laser pulse was measured with a calibrated joulemeter. The energy of incident laser pulses was 15 mJ, which yielded an incident laser fluence of 19 mJ cm^{-2} . The pulsed laser radiation with such parameters induced an insignificant temperature rise ($<10^{-2}\text{ }^{\circ}\text{C}$) in the samples. The pulsed laser radiation was delivered to the samples from above with the use of a prism. In the experiments with aqueous, absorbing solutions of potassium chromate we used the third harmonic of the Nd:YAG laser ($\lambda = 355\text{ nm}$).

Wide-band PVDF acoustic transducers (0.1–5 MHz) were used for OA pressure wave detection. Although the transducers were heated, the sensitivity of the PVDF piezoelectric film does not depend on the temperature that yields accurate OA pressure wave measurements.

A photodiode (Det 210, Thorlabs, Inc.) was used to monitor the total diffuse reflectance (R_d) from samples. Since the photodiode was positioned at a large distance from the sample, the photodiode signal was proportional to R_d .

The averaging and recording time for one signal was 1 s. The repetition rate of the pulsed laser radiation was 10 Hz and allowed for averaging of 10 pressure wave profiles during this time. The pressure profiles were recorded by a digital scope (TDS 520, Tektronix Inc.) and in a computer.

Tissue heating was performed using hot air from a heatgun (figure 1). Tissue samples were placed on the transducer and covered by a quartz plate of diameter 40 mm and thickness 10 mm to avoid direct contact with hot air.

We performed slow and rapid heating of the samples by varying the distance between the heatgun and the sample surface. Slow heating ($\approx 2\text{ }^{\circ}\text{C min}^{-1}$) produced a uniform temperature distribution in the samples and was used to monitor changes in optical properties during heating. Rapid tissue heating by hot air ($\approx 1\text{ }^{\circ}\text{C s}^{-1}$) was used to obtain a one-dimensional temperature distribution (gradient) in tissue. Conductive heating through the quartz plate provided a plane temperature front in the sample parallel to the transducer surface. Tissue samples were also covered with a plastic film from other sides to avoid dehydration and lateral heating.

A multi-sensor temperature probe with a variable number of thermocouples (from two to four), connected to a temperature reading device (Scanner Plus, Azonix Inc.), was inserted into the samples to provide temperature measurements simultaneously at different depths with an accuracy of about $0.3\text{ }^{\circ}\text{C}$. The use of the multi-sensor probe allowed for reconstruction of the actual temperature gradient, which was compared with the OA temperature mapping.

2.2. Samples

The experiments were performed with absorbing aqueous solutions of potassium chromate (K_2CrO_4), gelatin phantoms, and freshly excised canine liver (five samples) and myocardium (five samples). The tissue samples had dimensions of about $50 \times 50 \text{ mm}^2$ and a thickness of 10–30 mm.

The aqueous solution of potassium chromate served as a model object with thermophysical properties close to that of pure water, but with absorption in the UV. Distilled water for solution preparation was boiled for 15 min to avoid air bubble formation during the heating. The absorption coefficient of the solution calculated from the optoacoustic pressure profile by exponential fitting was 11 cm^{-1} .

To simulate tissue, we used a gelatin phantom of cylindrical shape with a radius of 60 mm and a thickness of 30 mm. India ink was used as an absorber in the phantoms to obtain an absorption coefficient of 4 cm^{-1} , typical of tissues in the NIR spectral range (Welch and van Gemert 1995).

2.3. Theoretical background

The thermal expansion of an absorbing medium heated by a short laser pulse with an incident laser fluence, F_0 , induces a pressure rise, $P(z)$, in the irradiated volume upon stress-confined irradiation conditions (Gusev and Karabutov 1993):

$$P(z) = \left(\frac{\beta c_s^2}{C_p} \right) \mu_a F(z) = \Gamma \mu_a F(z) = \Gamma \mu_a F_0 e^{(-\mu_a z)}, \quad (1)$$

where β [1°C^{-1}] is the thermal expansion coefficient, c_s the speed of sound, C_p [$\text{J g}^{-1}^\circ\text{C}^{-1}$] the heat capacity at constant pressure, $F(z)$ [J cm^{-2}] the laser fluence and μ_a [cm^{-1}] the absorption coefficient of the medium. The expression $\beta c_s^2 / C_p$ in equation (1) represents the Grüneisen parameter, Γ (dimensionless). The factor $e^{(-\mu_a z)}$ represents exponential attenuation of the optical radiation in the medium. Therefore, the OA pressure is dependent on Γ , the fluence, and the optical properties of the medium. Equation (1) is valid under the condition of stress-confinement when pressure relaxation is negligible during the heat deposition. The stress-confined condition can be achieved in tissues by using nanosecond optical pulses.

The Grüneisen parameter of water is temperature dependent (Burmistrova *et al* 1979, Sigrist 1986). These studies demonstrated the linear dependence of the OA pressure amplitude on temperature in water and, thus, can be expressed by an empirical equation:

$$\Gamma = A + BT, \quad (2)$$

where A and B are constants and T is the temperature. One can rewrite equation (1) in the case of an absorbing medium as

$$P(z) = (A + BT(z)) \mu_a F_0 e^{(-\mu_a z)} \quad (3)$$

and in the case of strongly scattering media in deep (not in the subsurface) areas as

$$P(z) = (A + BT(z)) k \mu_a F_0 e^{(-\mu_{\text{eff}} z)}, \quad (4)$$

where $T(z)$ is the temperature distribution in tissue, k the parameter resulting from multiple scattering in tissue and is

dependent on the absorption and scattering coefficients (Welch and van Gemert 1995) and μ_{eff} is the effective attenuation coefficient.

Rearranging equation (4), one can obtain

$$T(z) = C + \frac{D \cdot P(z)}{P(z)_{T=T_0}}, \quad (5)$$

where $P(z)_{T=T_0}$ is the OA pressure profile recorded at the initial temperature T_0 , and C and D are parameters that are dependent on tissue properties. Therefore, by recording and analysing the temporal OA pressure profile, one can reconstruct the temperature distribution during hyperthermia.

3. Results

Figure 2 shows typical OA pressure profiles recorded from the aqueous solution of potassium chromate from 25°C to 70°C . The amplitude of the OA pressure signal recorded at the temperature of 70°C was approximately 2.8 times greater than that at the temperature of 25°C . A noticeable difference between the amplitude of OA pressure profiles recorded at 37°C and 43°C was also detected. The temporal profiles did not change significantly during heating and cooling. The OA pressure amplitude induced in the canine liver versus temperature from 36°C to 50°C , and the OA pressure amplitude induced in the canine myocardium versus temperature from 28°C to 55°C are shown in figures 3(a) and (b), respectively. It is clearly seen that the amplitude increases linearly with the temperature. The relative increase in pressure amplitude was approximately 1.5% per degree centigrade, which results in about 9% increase in the liver tissue and 4% increase in the myocardium tissue heated from 37°C to 43°C . This temperature rise is normally applied for hyperthermia. The optical properties of liver tissue were constant during heating, up to the temperature of coagulation (50 – 55°C), and dramatically changed due to protein denaturation at higher temperatures (data not shown).

The Grüneisen parameter of water and the OA pressure amplitude induced in the aqueous solution are presented versus temperature in figure 4. The Grüneisen parameter of water was theoretically calculated from the data on the thermal expansion coefficient, speed of sound and heat capacity at

Figure 2. OA pressure profiles induced in aqueous solution at various temperatures.

Figure 3. Amplitude of OA pressure induced in canine liver (a) and canine myocardium (b) versus temperature. The vertical and horizontal lines in (a) mark the temperature range that is normally used in tumour hyperthermia.

Figure 4. Theoretically calculated water Grüneisen coefficient and optoacoustic pressure amplitude induced in aqueous solution as a function of temperature.

constant pressure reported in Weast (1974) and Kikoin (1976). The Grüneisen coefficient has an almost linear dependence on temperature. There is a good correlation between the theoretical and experimental data.

Figure 5 presents the optical effective attenuation coefficient and total diffuse reflectance versus temperature recorded in the canine liver during slow conductive heating and passive cooling of the tissue samples (figures 5(a) and

Figure 5. Optical attenuation coefficient (a) and total diffuse reflectance (b) versus temperature during slow conductive heating in canine liver. The solid line and solid circles indicate heating, while the dashed line and open circles correspond to the cooling of the tissue samples.

(b), respectively). No changes in the attenuation coefficient from 25°C to about 53°C were observed in these experiments. However, the OA pressure amplitude induced in the canine liver increased linearly with the temperature from 36°C to 50°C (figure 3(a)). Therefore, only the Grüneisen parameter of liver increased with temperature, while the optical properties did not change. These data experimentally confirm the hypothesis that the Grüneisen parameter of tissue increases with temperature.

OA pressure profiles used for temperature distribution reconstruction recorded at different times during fast heating of liver and gelatin samples are presented in figures 6(a) and (b), respectively. Calibration graphs were plotted for liver and gelatin samples by using the temperature recorded at the sample surface by the first thermocouple of the multi-sensor temperature probe. The constants C and D were found from these graphs by fitting of $P(T)$ graphs for these samples. By using these constants and equation (5), we reconstructed temperature distributions from the recorded pressure profiles. The reconstructed temperature distributions in the liver and gelatin samples are shown in figures 7(a) and (b), respectively. They were compared with the actual temperature recorded by the multi-sensor temperature probe (the circles indicate actual temperature readings from the multi-sensor temperature probe). The optoacoustically reconstructed temperature from figures 7(a) and (b) versus

Figure 6. OA pressure profiles recorded from canine liver (a) and gelatin phantom (b) at different times during heating.

the actual temperature measured with thermocouples is shown in figure 8. These results demonstrate good agreement between the temperature distributions reconstructed using the OA technique and those measured using the multi-sensor temperature probe. The accuracy of temperature monitoring was less than 1°C , while the spatial resolution was about 1 mm.

4. Discussion

Results of the experiment with the aqueous solution of potassium chromate demonstrated good agreement of the temperature dependence of the measured OA pressure amplitude with the Grüneisen parameter of water calculated theoretically from the literature data. The increase of the Grüneisen parameter of water with temperature is caused mostly by the increase in the thermal expansion coefficient β (equation (1)). The slight deviation of the experimental data points from the theoretical curve observed in figure 4 can be explained by the differences between the thermophysical properties of the aqueous solution of potassium chromate and pure water.

The increase in the Grüneisen parameter of the investigated tissues with temperature is less than that in water. The difference in temperature dependence of the

Figure 7. Reconstructed temperature profiles in canine liver (a) and gelatin phantom (b). Circles indicate actual temperature in the samples recorded with the multi-sensor temperature probe.

Figure 8. Temperature calculated from OA signals versus actual temperature measured with the multi-sensor temperature probe for data shown in figure 7: R —correlation coefficient; P — p -value; SD —standard deviation of data points.

Grüneisen parameters of water, liver and myocardium was due to the composition of the tissues. The tissue thermal expansion coefficient and speed of sound are less dependent on temperature than those corresponding to water (Duck 1990).

The proportions of water, lipid and proteins vary in tissues, and this yields different thermal expansion coefficients, speeds of sound, and heat capacities, as well as different dependences of these parameters on temperature. It is likely that the lipid content is a major factor because the thermal expansion coefficient and speed of sound in fat decrease with temperature (Duck 1990). This explains the differences in the temperature dependence of the Grüneisen parameter of water, liver and myocardium, because the lipid content in liver and myocardial tissue is 4.6% and 6.2%, respectively (Duck 1990).

The results obtained demonstrated that the attenuation coefficient and total diffuse reflectance of tissue do not change during moderate heating in the temperature range usually applied for hyperthermia. This means that absorption and reduced scattering coefficients do not significantly change in this temperature range.

Data from (Gertner *et al* 1997, Clarke *et al* 2003) suggest a slight decrease in the ultrasound attenuation coefficient (UAC) for temperatures under 50°C in tissues. Therefore, one could expect minimal influence of the temperature UAC dependence on the optoacoustic signals detected at temperatures of up to 50°C. The temperature dependence of UAC can be taken into account for more precise OA measurements during thermotherapy.

Although some data reported in the literature (Laufer *et al* 1998) indicate that the tissue reduced scattering coefficient μ'_s may increase (about 0.5% per degree centigrade) for temperatures below 50°C, no measurable changes in the effective attenuation coefficient and total diffuse reflectance were observed in our studies (figures 5(a) and (b)). This variation in results can be explained by the differences between the tissue types used.

The linear dependence of the OA pressure amplitude on temperature obtained in our experiments was used for temperature mapping in liver and gelatin samples. As a result, we were able to monitor the temperature change in tissues by detecting the pressure wave and comparing it with the pressure wave recorded before the heating. An accurate reconstruction of temperature distribution in tissues requires calculation of the parameters C and D from equation (5). In general, these parameters can be calculated before or during thermotherapy. The parameters C and D could be calculated during thermotherapy by measuring the temperature at one point, for instance, on the tissue surface and comparing it with the pressure signal induced at this point. In our studies, we used one thermocouple placed on the sample surface for calibration. Another possible method of obtaining parameters C and D is to monitor the temperature from the surface using the infrared thermography technique.

The results shown in figures 7 and 8 indicate that one can monitor the temperature rise in tissues by detecting the pressure wave amplitude with an accuracy of better than 1°C at depths of up to 3 cm from the transducer surface. The standard deviation of data points was equal to 0.57°C. The accuracy of tissue temperature measurements was limited in our experiments by the 3% instability of averaged (10 times) laser pulse energy. Current laser systems with stabilized pulse energy available in the market have 1% stability; therefore, a higher accuracy of temperature measurement could be achieved.

5. Conclusion

Our studies demonstrated that the OA technique can be used: (1) to detect temperature changes in tissue with an accuracy of better than 1°C; (2) to perform temperature measurements in real time; (3) to provide temperature measurements at depths of up to several centimetres; and (4) to reconstruct the temperature distribution in tissues during heating. However, it is necessary to note that the studies in tissues were performed *in vitro*. The influence of different physiological parameters during tissue heating *in vivo* (such as increased tissue perfusion) on the accuracy of OA temperature monitoring should be evaluated in additional studies. However, one can expect a limited influence of these parameters, *in vivo*, on the accuracy of temperature monitoring with the OA technique at moderate (a few degrees) heating.

Since OA wave generation and detection are not influenced by the nature of the heating agent, this technique can potentially be applied for real-time temperature mapping in tissues during heating by radiofrequency, microwave, ultrasonic or laser radiation. In addition, optoacoustic monitoring is a non-invasive technique because low-energy laser pulses applied for pressure wave generation cannot cause thermal or mechanical damage to tissues. Therefore, optoacoustic monitoring of temperature has the potential to become a valuable real-time feed-back modality for thermotherapy.

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