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Structural changes in connective tissues caused by a moderate laser heating

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Abstract. **The structural changes in adipose and fibrous tissues caused by 2- and 3-W IR laser irradiation are studied by the methods of IR and Raman spectroscopy and differential scanning calorimetry. It is shown that heating of fibrous tissue samples to 50 °C and adipose tissue samples to 75 °C by IR laser radiation changes the supramolecular structure of their proteins and triacylglycerides, respectively, without the intramolecular bond breaking. Heating of fibrous tissue to 70 °C and adipose tissue to 90–110 °C leads to a partial reversible denaturation of proteins and to oxidation of fats.**

Keywords: *laser thermoplastics, connective tissues, collagen denaturation, melting of triacylglycerides.*

1. Introduction

The use of new laser technologies in medicine has stimulated extensive studies of the physicochemical processes induced by laser radiation in biological tissues [1]. Biological tissues are irradiated by moderate-power lasers, which do not produce any irreversible chemical destruction of the macromolecules constituting the frame of the tissue matrix, but cause a therapeutic or surgical effect (commissure of blood vessels [2], reshaping of the nasal septum cartilage [3], etc.).

Real-time measurement of the optical characteristics of biological tissues allows us to estimate the variations induced by laser radiation in these tissues [3–5]. However, such data are not sufficient to provide a description of all physicochemical processes proceeding in this case. At the same time, a change of the supramolecular structure of the biological tissue leads to a variation of its biomechanical, rheological, optical and other properties.

The aim of this work is to study the possible variations in the chemical composition and structural organisation of the connective tissue components caused by heating by the IR laser radiation.

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2. Materials and methods

We studied the fibrous tissue (FT) and subcutaneous fat extracted from the zygomatic region of a six-month old pig, as well as the subcutaneous fat from the lower eyelid and thigh regions of a human being. The materials were stored at 4 °C in a refrigerator and used over a period of five days. We found that the physicochemical properties of tissues did not change over this period. The fibrous tissue was successively washed in water and kept in acetone at 4 °C for 12 hours and in diethyl ether at 20 °C for four hours. This resulted in a complete extraction of lipids from the tissue.

The samples were irradiated by an LS-1.56-5 IR erbium-doped fibre laser (IRE–'Polyus' group, Russia) at a wavelength of 1.56 μm. The fibrous and adipose tissues were irradiated in various regimes. Vertically held FT samples were irradiated through an optical fibre. The laser power was 2 and 3 W, and the power density on the samples was 7 and 10 W cm^{-2}, respectively, for a 6-mm diameter of the exposed region. The temperature was monitored by remote IR radiometry technique. The IR emission signal was collected from a 2.5-mm^2 area. The samples were heated up to 50 and 70 °C for a radiation power of 2 and 3 W, respectively.

Upon irradiation of adipose tissue samples, the laser spot diameter was varied from 3 to 7 mm by varying the distance between the end of the optical fibre and the sample surface. The irradiation time varied from 30 to 270 s for a radiation power of 3 and 3.5 W. The sample temperature was measured with a needle thermocouple with a tip of diameter 30 μm.

All the spectral characteristics of the tissues were measured with an EQUINOX 55/S Fourier spectrophotometer with an attachment for Raman scattering. The absorption spectra of adipose tissue samples deposited on a NaCl substrate were recorded with a resolution of 0.5 cm^{-1}. Opaque FT samples were dried over P_2O_5, pressed between two CaF_2 windows, and their Raman spectra were recorded. The Raman spectrum excited by a 1.064-μm, 0.2-W Nd:YAG laser was recorded with a resolution of 4 cm^{-1}, averaged over 150 scans, and subjected to inverse Fourier transformation. Each spectrum was calibrated by the intensity of C–H vibrations (2932 cm^{-1}) and subjected to Fourier smoothing over four points.

The thermal behaviour of the samples was studied using a Mettler TA4000 differential scanning calorimeter. The samples, weighing not more than 15 mg each, were analysed in hermetic aluminium boxes of volume 0.04 cm^3 each, their

heating and cooling rates being 10 °C min^{-1} and 5 °C min^{-1}, respectively. The signal was calibrated to the heat of melting of indium.

3. Results and discussion

3.1 Fibrous tissue

Fig. 1 shows the Raman spectra of a fibrous tissue. Such spectra are typical for connective tissue variety containing collagen [6–8]. No new bands appear in the region of C–H vibrations in the spectrum of the irradiated FT, which shows that no chemical destruction of the tissue occur upon irradiation. The relative intensity of the Amide III band decreases after irradiation of the tissue, which indicates a change in the supramolecular structure of the proteins.

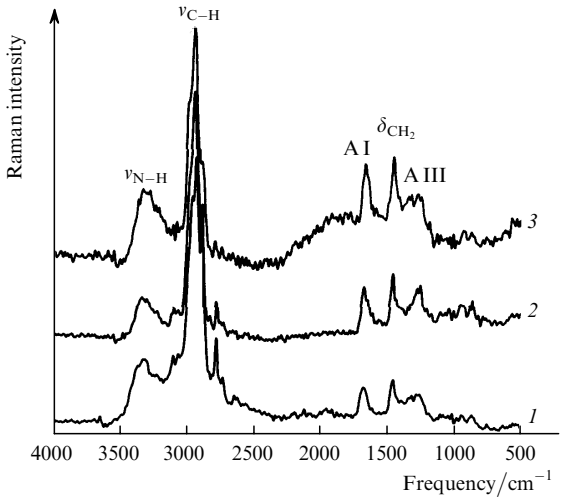

Figure 1. Raman spectra of fibrous tissue for the test sample (*1*) and samples exposed to laser radiation of power 2 (*2*) and 3 W (*3*); ν_{C-H}, ν_{N-H} are the frequencies of valence vibrations, δ_{CH_2} is the frequency of deformation vibrations; AI and AIII are characteristic vibrational bands of the peptide group.

Fig. 2 shows the results of calorimetric studies of the FT samples (containing 70 % water by weight). The thermograms of test samples at the temperature $T_{m1} = 70$ °C display a distinct peak characterising an endothermic transition with a thermal effect Q_{m1} equal to 46 ± 2 J per gram of the dry residue of the biological tissue. The temperature T_{m1} and the thermal effect Q_{m1} of the transition correspond to the parameters of collage denaturation in the connective tissue samples with a water content of about 70 % [9–11]. Laser heating of the FT to a temperature of 50 °C does not lead to a denaturation of collagen, and the endothermic effect is reproduced in subsequent calorimetric investigations (curve *2*). These data confirm that the structure of collagen fibre is preserved after irradiation by a 2-W laser for 10 s. The shape of the thermograms changed significantly after heating the samples to 70 °C and above (curve *3*). The endothermic peak corresponding to the collagen denaturation either vanished or strongly broadened, while the value of Q_{m1} decreased by several times. This means that the collagen denaturation occurred after laser irradiation.

Thermograms of the samples exhibit two more peaks

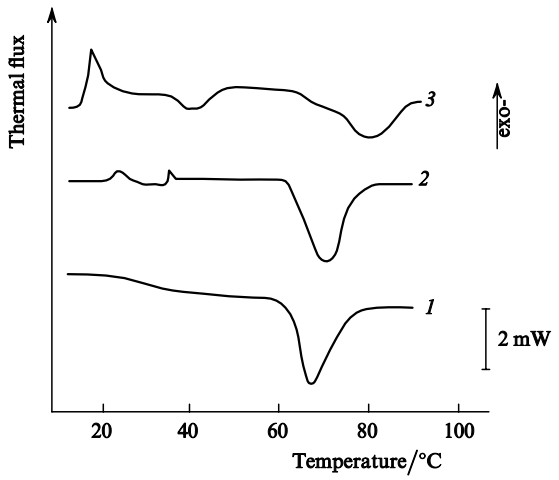

Figure 2. Thermograms of the fibrous tissue for the test sample (*1*) and samples exposed to laser radiation during 10 s and heated to $T_{max} = 50$ (*2*) and 70 °C (*3*).

corresponding to exothermic ($T_r = 21$ °C, $Q_r = 9.2$ J g^{-1}) and endothermic ($T_{m2} = 42.5$ °C, $Q_{m2} = 7.1$ J g^{-1}) transitions. Such a thermal behaviour of the samples is typical of annealed polymers and is explained by recrystallisation of an amorphous polymer at a temperature higher than the glass-transition temperature, followed by melting of the crystalline regions [12].

Similar to the effects described above, the IR laser heating resulting in denaturation (destruction of the triple helix structure) of collagen macromolecules is followed by a rapid cooling down to 18°C, when the segmentary mobility of polypeptide chains is insufficient for their packing into a quasi-crystalline fibrous structure. Subsequent heating leads to an increase in the mobility of parts of the macromolecule and a triple helix structure (microfibril) is formed again. However, this process proceeds only partially ($Q_{m2} < Q_{m1}$), and the collagen fibre is not restored ($T_{m2} < T_{m1}$).

Note that the collagen melting at 40 °C was also observed during repeated temperature scanning after a certain time interval (no less than an hour after the first heating) [11]. However, the process of recrystallisation during repeated heating of the collagen is not described in the literature. The result obtained in this work can be explained by high rates of heating and cooling upon IR laser irradiation. It seems that during rapid heating of collagen molecules (the main component of the FT), their polypeptide chains do not have time to come apart completely and to form random coils. This facilitates a repeated packing of the chains into a triple helix during subsequent heating after an almost instantaneous cooling, which leads to 'freezing' of the chain mobility.

3.2 Adipose tissue

The absorption spectra of the human and pig adipose tissue (Fig. 3) exhibit vibrations inherent in esters to which triglycerides, the main components of the adipose tissue, belong [13]. The absorption intensity in the region of amide bands is low, which corresponds to a low concentration of proteins. The spectra of test samples and samples of human and pig adipose tissues heated by laser radiation to a temperature of 75 °C were found to be identical, i.e., laser radiation did not cause any noticeable change in the chemical composition of adipose tissues. A decrease in the

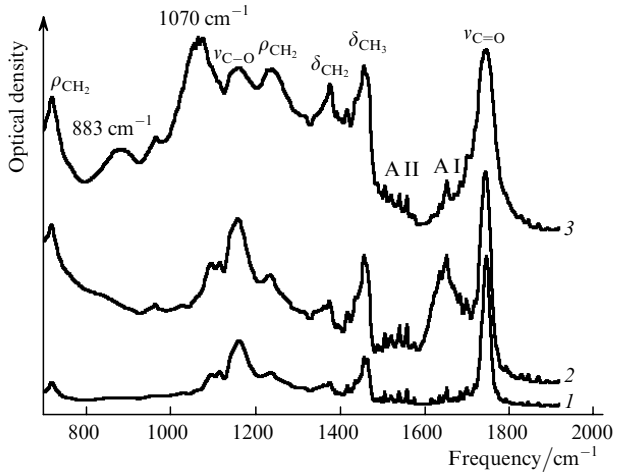

Figure 3. Absorption spectra of the adipose tissue of a pig (*1*) and the adipose tissue of a human being for the test sample (the shape of the spectrum remained unchanged after laser heating to 75 °C for two minutes) (*2*), and a sample heated by the laser up to 90–110 °C for one minute (*3*); ρ_{CH_2} is the frequency of torsional vibrations (see also the notation in Fig. 1).

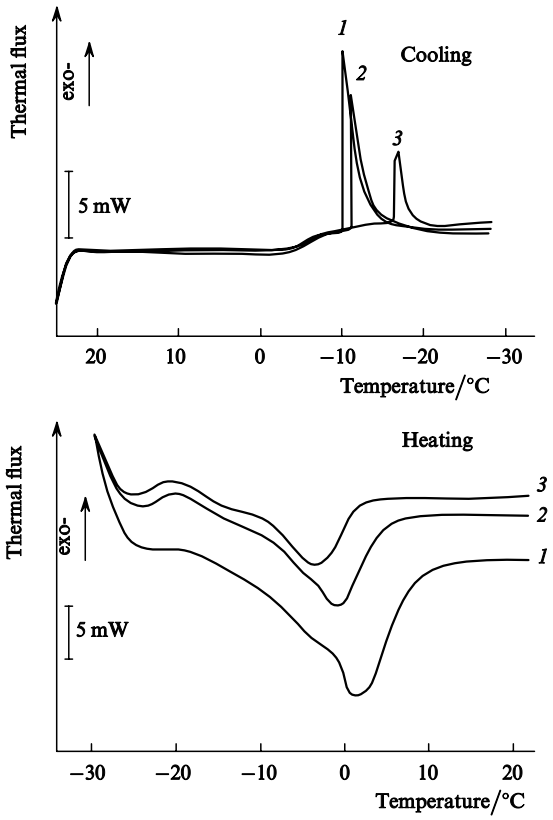

Figure 4. Thermograms of the human adipose tissue for the test sample (*1*) and samples heated by laser radiation for 90 s to 50 (*2*) and 70 °C (*3*).

relative intensity of the Amide I band can be caused by the destruction of the native conformation of proteins [8].

A more intense IR laser irradiation (exposure time 60 s, sample heating temperature 100–130°C) leads to distinct variations in the Raman spectrum of the human adipose tissue. The 1070- and 883-cm^{-1} absorption bands appear, which correspond to the vibrations of the ester and epoxy groups, respectively [13]. The relative intensity of the 3006-cm^{-1} absorption band, corresponding to the C–H vibrations at the C=C double bond, decreases. We assume that a high-intensity laser heating of the open regions of the adipose tissue causes an oxidation of the double bond by oxygen from the air.

The study of the thermal characteristics of the adipose tissue showed that an increase in the maximum temperature T_{max} and time τ of laser treatment resulted in a decrease in the crystallisation temperature T_{cr} and the temperature T_m of the subsequent melting, as well as the melting heat Q_m (Table 1). Moreover, the melting of irradiated samples was preceded by an exothermic transition with the thermal effect Q_{ph} at the temperature T_{ph} (Fig. 4). This effect was not observed in the pig adipose tissue, which melts at a temperature below 45 °C.

Obviously, the difference in the thermal behaviour of the human and pig adipose tissues is due to a difference in the composition of their triglycerides. An increase in the residual fraction of unsaturated fatty acids in triglycerides reduces the melting point of the human adipose tissue [14, 15]. Laser irradiation provides heating of the human adipose tissue to temperatures considerably higher than its melting point.

The melts of fats are known to retain elements of the ordered structure (molecular associates), which serve as crystallisation centres during subsequent cooling of the melt. Laser heating of the human adipose tissue to 50–70 °C leads to a considerable superheating of the triglycerides melt, resulting in a decrease in the size of molecular associates and their destruction. A significant decrease in T_{cr}, T_m and Q_m with increasing τ and T_{max} indicates a lower stability of the crystalline phase formed during cooling of the adipose tissue irradiated by the laser, which is explained by an increase in the residual fraction of triglycerides in the liquid state upon supercooling. It has been suggested [14–

Table 1. Change in thermal characteristics of adipose tissue after laser treatment.

Adipose tissue	τ/s	T_{max}/°C	T_{cr}/°C	Exothermic transition		Endothermic transition	
				T_{ph}/°C	Q_{ph}/J g^{-1}	T_m/°C	Q_m/J g^{-1*}
Human	0	–	−7.5 ± 1.5	–	–	1.2 ± 0.3	106 ± 8
	30	50	−8.5 ± 0.5	−20.3 ± 0.2	3.2 ± 0.5	−0.6 ± 0.3	95 ± 10
	90	50	−10.9	−20.3	4.7	−1	100
	270	50	−14.2	−20.3	4.4	−1.8	86
	30	70	−16 ± 1	−20.3 ± 0.1	4.8 ± 1.2	−3.5	68 ± 8
Pig	–	–	−2.2 ± 0.8	–	–	29.4 ± 0.5	95 ± 10
	30	70	−2.8	–	–	30 ± 0.5	93 ± 10

*The melting heat of triglycerides in the adipose tissue of test samples corresponds to the melting heat Q_m of natural fats [14, 15].

16] that a rapid cooling of the superheated human adipose tissue results in the formation of a metastable hexagonal crystalline modification of triglycerides. Subsequent heating transforms the hexagonal modification to a more stable orthorhombic modification. The exothermic effect corresponding to this transition is manifested clearly in the thermograms of the irradiated samples, which is followed by melting of the crystalline phase.

A rapid local laser heating of the pig adipose tissue to 70 °C does not cause a destruction of the molecular associates. This is confirmed by the identical thermal behaviour of the initial and irradiated samples.

The endothermic process associated with the denaturation of proteins was observed in the thermograms of the human and pig adipose tissue samples. The denaturation peak temperatures in these cases were 69.3 and 66.9 °C, respectively. The thermal effect of the protein denaturation process decreases after laser treatment of the initial adipose tissue. Thus, laser heating of the adipose tissue results in denaturation of proteins.

4. Conclusions

A moderate laser heating, which does not affect the intramolecular bonds of the components of adipose and fibrous tissues, leads to a significant rearrangement of their supramolecular structure. In this case, a destruction of the molecular triglyceride associates and a denaturation of proteins can be observed in human adipose tissues. An increase in the laser radiation power causes an overheating of the tissue and stimulates thermochemical processes that affect the molecular structure as well. The collagen fibres in triglycerides melt if the temperature increases above 70 °C upon laser irradiation. However, the triple helix structure of the collagen macromolecules may be restored partially.

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