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Measurement of the temperature dependence of Young's modulus of cartilage by phase-sensitive optical coherence elastography

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Abstract. **The development of an effective system to monitor the changes in the elastic properties of cartilage tissue with increasing temperature in laser reconstruction is an urgent practical task. In this paper, the use of phase-sensitive optical coherence elastography for detection of elastic waves in the sample has allowed Young's modulus of cartilage tissue to be measured directly during heating. Young's modulus was calculated from the group velocity of propagation of elastic waves excited by means of a system supplying focused air pulses. The measurement results are in agreement with the results of measurements of the modulus of elasticity under mechanical compression. The technique developed allows for noninvasive measurements; its development is promising for the use *in vivo*.**

Keywords: *cartilage tissue, laser heating, measurement of the modulus of elasticity, optical coherence tomography.*

1. Introduction

The process of stress relaxation in cartilage tissue under laser heating is the basis for new methods of shape correction and regeneration of cartilage in otolaryngology and orthopaedics [1, 2]. Treatment conditions of laser action should provide a short-term decrease in the elastic modulus of tissue without its denaturation and destruction of cells. Currently, the safety of laser procedures on changing the shape of the nasal septum cartilage is provided by the control system based on measuring the temperature of irradiated tissue at the periphery of the laser action [3], whilst the control system for laser reconstruction of the intervertebral discs is based on measuring the intensity of scattered laser light [4]. However, since the stress field is not measured directly, the stress relaxation cannot commonly be provided completely, so that the presence of residual stresses causes subsequent tissue deformation, reduces the effectiveness of laser procedures and in some cases leads to the need for reoperation. Therefore, the development of new control methods based on direct monitoring of elastic characteristics of cartilage during laser irradiation represents a very urgent task [5].

Some works are devoted to determination of the temperature dependence of the elastic properties of cartilage using the method of irreversible compression [6]. During the measurements, cartilage tissue was heated to a certain temperature, after which a mechanical stress was applied to a sample. With the passage of time, the stress relaxation occurred under the action of the cartilage tissue compression. Young's modulus of cartilage was determined after the relaxation of the external stress. However, when using this method, tissues undergo irreversible changes, which makes it impossible to use the method for monitoring the elastic properties of cartilage in vivo. Also, in some works, by means of excitation of free and forced mechanical oscillations, the dynamic modulus and internal friction of cartilage tissue were measured during the laser-induced stress relaxation [7]. For the diagnosis of the cartilage tissue properties, optical methods are being actively used. For example, second-harmonic generation and two-photon fluorescence microscopy have been successfully used for imaging the intercellular structure of healthy and damaged articular cartilage tissue [8, 9]. Analysis of the angular distribution of the light scattered by the sample enables one to diagnose the type of the cartilage tissue, which can be useful in detection of various diseases [10].

Known are the works on studying the relaxation of the external stress in cartilage tissues by means of the backscattering method [11, 12]. Simultaneous measurements of the temperature, internal stress and intensity of backscattering of light during laser heating of the sample have revealed that, at a certain temperature, cartilage tissue undergoes a phase transition. The transition temperature value is dependent on the heating rate of the sample [13]. The intensity of backscattering of light is related to the temperature of cartilage tissue, which allows employing the intensity magnitude as the sensor of the sample temperature during heating.

To measure directly the temperature dynamics of the elastic properties of cartilage tissues, it seems promising to use the method of elastometry based on optical coherence tomography (OCT) [14–16]. This method for measuring the longitudinal and transverse elastic moduli of the sample consists in creating a mechanical excitation in tissue and evaluating the parameters of its propagation by means of phase-sensitive OCT. The excitation can be caused in many ways, e.g. by mechanical forces [16, 17], acoustic force [18], or by the action of laser pulses [19].

A method for measuring Young's modulus of biological tissues via the excitation of elastic waves on the sample sur-

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face under the action of focused air pulses has been recently reported [20]. This method was applied to study the elastic properties of cancer tumours [21] and the structures that mimic soft tissues [22]. Its advantage is that it is promising for use *in vivo* (e.g., the method has been used to measure the elastic properties of the mouse cornea *in vivo* [23]). It is also worth of note that phase-sensitive OCT allows detection of mechanical perturbations with the amplitude of several microns, and so there is no need in significant effect on the tissue under studying.

The present work is dedicated to developing a method for measuring the temperature dynamics of Young's modulus of cartilage tissue by means of noninvasive elastometry based on phase-sensitive OCT. The method developed will allow the researcher to monitor the changes in the elastic properties of cartilage tissue during the heating process. The approach proposed in this work has the prospects of clinical application to oversee the process of laser reconstruction of cartilage tissues.

2. Materials and methods

A fresh cartilage of the nasal septum of a pig was obtained immediately after the animal's euthanasia at a local slaughterhouse. The cartilage was separated from the bones and soft tissues, and the rectangular samples were cut out of it. To heat cartilage tissue, a saline with 0.9% NaCl mass concentration, being heated, was used. A rectangular plate of 3-mm-thick cartilage tissue measuring 1×1.5 cm was placed in a reservoir with saline and mounted horizontally in such a way that its lower half was immersed into the liquid while the upper part remained in the air (see Fig. 1). The salt solution in the vessel was warmed by means of a heating element; the measurements were carried out at a temperature varied from 20 to 70 °C. The temperature was monitored using a thermocouple embedded into the sample to a depth of about 0.5 mm, the measurement error constituted 0.05 °C. The temperature at the beginning of the measurement process was referred to as the sample temperature; the temperature fluctuations did not exceed 1 °C in the course of the measurements.

During the experiment, elastic waves were excited in the sample. The method of phase-sensitive OCT allowed measuring the group velocity of elastic waves propagation, which was used to calculate Young's modulus of tissue under study. Elastic waves were excited by means of the air pulse generation system, capable of producing a concentrated air flow of less than 1 ms in duration. The system consists of a balloon with air under high pressure and a control block. An additional signalling channel of the control block ensures the synchronisation of the pulse signal generation with the recording of the signal by OCT. The pressure in the air source is measured with a manometer; the air pulse comes out of a needle of 150 μm in diameter with a flat edge. The spatiotemporal profile of the air pulse has a Gaussian shape. The location of a point on the sample surface, close to which the air pulse is generated, can be precisely set using a three-coordinate linear micrometric feed. The air pulse creates a deformation in tissue; this system allows a contactless excitation of elastic waves in the sample.

For elastic waves to be detected, the method of phase-sensitive OCT is used. The experimental setup to study the elastic properties of cartilage tissue is shown in Fig. 1. The radiation source is a HSL2000 laser (Santec Inc., USA) tunable within a broad band (150 nm) with a centre wavelength of 1310 nm. The scan speed throughout the range of operat-

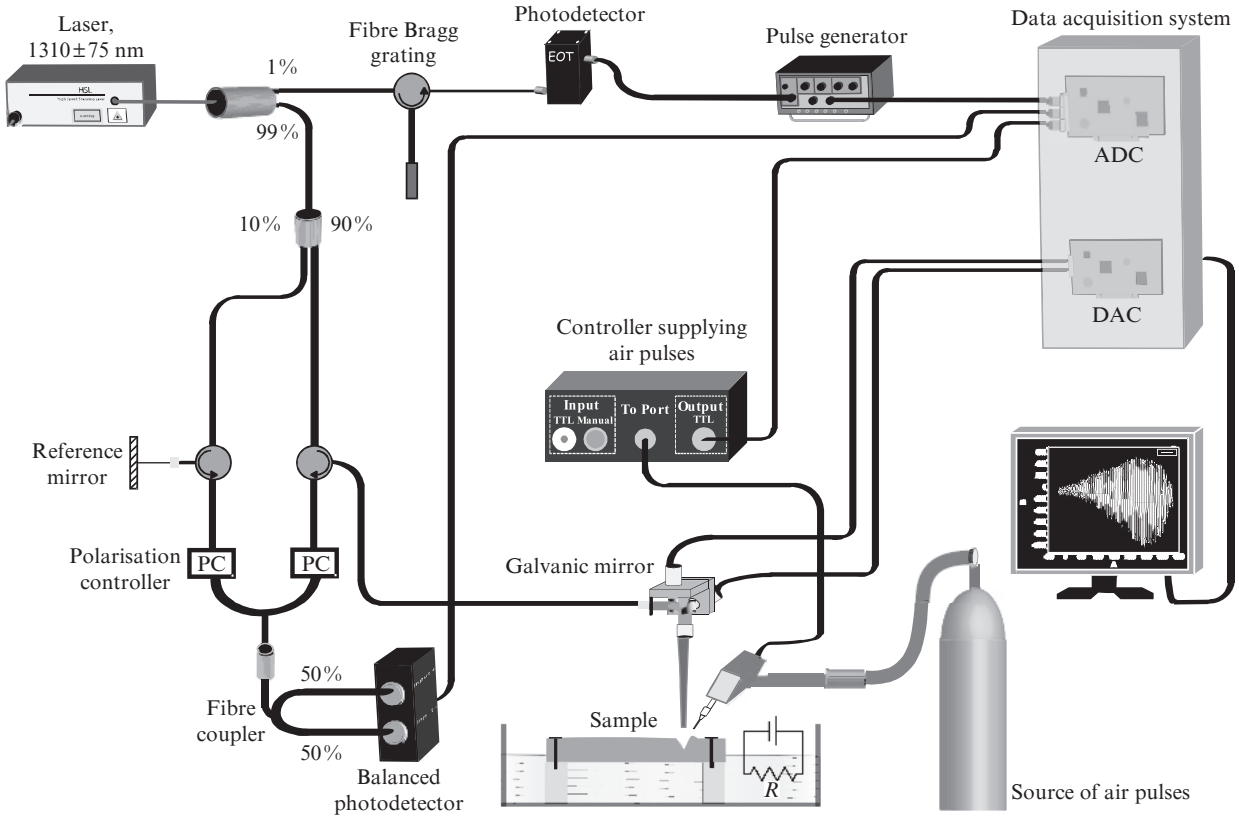

Figure 1. Experimental setup for measuring the temperature dependence of Young's modulus of cartilage tissue.

ing wavelengths amounts to 3×10^4 s^{-1}, and the output power is 36 mW. For imaging, a Mach–Zehnder interferometer is used. The interference pattern is detected by a balanced photodetector and digitised by an ADC. A fiber Bragg grating is used to initiate the A-scan. The experimentally measured phase stability of the system is 16 mrad (which corresponds to an optical path of 3.3 nm in the air). For the implementation of B-scan, a galvanic mirror is employed. The system supplying the air pulses generates the output pulse signal, which is recorded by the ADC to carry out synchronisation. In order to detect the propagation of elastic waves, the BM-scan is carried out from the point of excitation (under the needle of the system supplying the air pulses) along the direction of the wave propagation. The measurements are performed at 251 points, the distance between the starting and ending points of the scan constituting 6.3 mm. At each scan point, a signal of the phase shift of the light scattered on the sample is recorded. The start of the phase profile recording is synchronised with the time instant of the air pulse arrival. The recording is made at each point within 0.1 s with a sampling frequency of 30 kHz. More detailed information about this procedure can be found in previous studies [21–23].

The phase shift of the reflected radiation at the selected point of the sample corresponds to the tissue displacement at this point, which occurs during the passage of the shear elastic wave. To calculate the displacement A from the phase shift Φ of the OCT signal, the expression [23] was used

$$A = \lambda(\Phi/2\pi), \tag{1}$$

where λ is the centre wavelength of the laser source.

The velocity c of the shear elastic wave was calculated from the relation

$$c = d/\tau, \tag{2}$$

where d is the distance between two points of the sample, and τ is the delay time of the elastic wave. The location of the starting point was selected at a distance of ~0.4 mm from the wave excitation point, and the time delay of the elastic wave pulse appearance was defined relative to each subsequent scan point. To determine the time delay of the elastic wave pulse, the cross-correlation function of the displacements at the primary and selected points was constructed:

$$B(\tau) = \langle A_0(t)A_n(t+\tau)\rangle, \tag{3}$$

where $A_0(t)$ is the displacement at the initial point, and $A_n(t)$ is the displacement at a given point versus time. The time τ at which the cross-correlation function reaches its maximum, corresponds to the time delay of the elastic wave pulse at the selected point relative to the initial one. The wave velocity at each point is calculated by formula (2). The values of the velocities obtained are averaged for all points of the range selected (approximately 1 mm in length and 150 μm in depth of the sample), and the average group velocity of elastic waves propagation is calculated. The upper limit of the selected range turned out located at a distance of 25 μm from the sample surface, and so the velocity values thus obtained correspond to the velocity of the elastic shear wave propagation within the cartilage tissue volume.

Assuming that cartilage tissue possesses homogeneous isotropic elastic properties, Young's modulus of tissue can be calculated using the expression [24]:

$$E = 3\rho c^2, \tag{4}$$

where ρ is the substance density. In order to calculate E, the experimentally measured group velocity of the elastic wave propagation was used.

To analyse the reliability of the data on Young's modulus of cartilage tissue, derived from the experiment, the elastic properties of tissue have been measured by means of a uniaxial mechanical elastomeric B-Spec 2200 system (Instron Inc., USA). In both cases, we used the samples prepared from the same nasal septum cartilage tissue of the pig. In the experiment, a rectangular plate of cartilage tissue had been fixed with two terminals, then the plate was automatically compressed and the elasticity modulus was measured. The maximal deformation was set at 0.03; this value was chosen as a limit to prevent the irreversible damage of the sample [2]. To increase the temperature, the sample together with terminals was dipped into a tank of saline. The solution was warmed by means of a built-in heating element; the measurements were performed at a temperature in the range from 20 to 65 °C. The temperature was monitored by a thermocouple in exactly the same way as in case of OCT measurements.

3. Results and discussion

Figure 2 shows the typical time dependence of the tissue sample displacement during the elastic wave propagation, measured at two points located at different distances from the point of the wave excitation. It can be seen that after the start of recording the elastic wave reaches the measuring point and causes the displacement of the tissue sample. The elastic wave amplitude decreases and the delay time increases with increasing distance from the point of excitation.

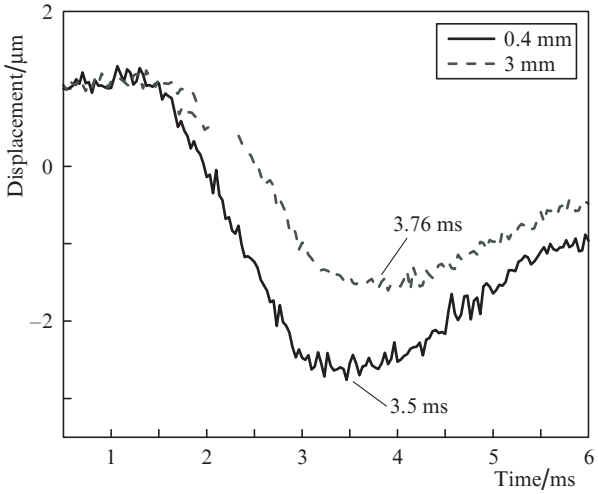

Figure 2. Displacement of the tissue sample vs. time during the elastic wave propagation. The measurements were made at distances of 0.4 and 3 mm from the point of wave excitation.

The dimensionless cross-correlation function of the signal of the tissue sample displacement is shown in Fig. 3. The function has a maximum at 0.26 ms – this is the time required for a wave to propagate over the distance of 2.6 mm between the measurement points.

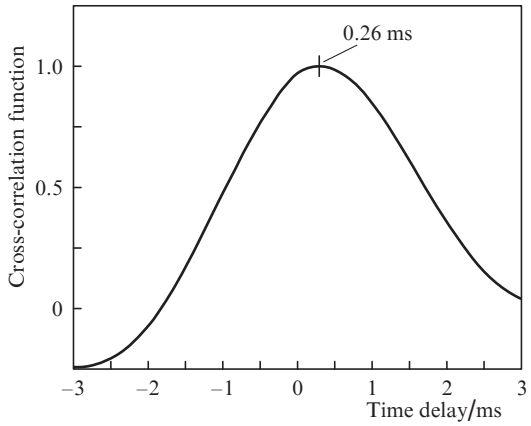

Figure 3. Dimensionless cross-correlation function of the tissue sample displacement during the elastic wave propagation, calculated at distances of 0.4 and 3 mm from the point of wave excitation.

Figure 4 shows a typical dependence of the velocity of elastic waves in the cartilage on the temperature in the range of 20–70 °C as measured by the OCT method. The velocity increases in the range of 20–40 °C, and then greatly reduces. This means that the elasticity of cartilage tissue increases at the beginning of heating, but when heated to 50 °C and higher – decreases.

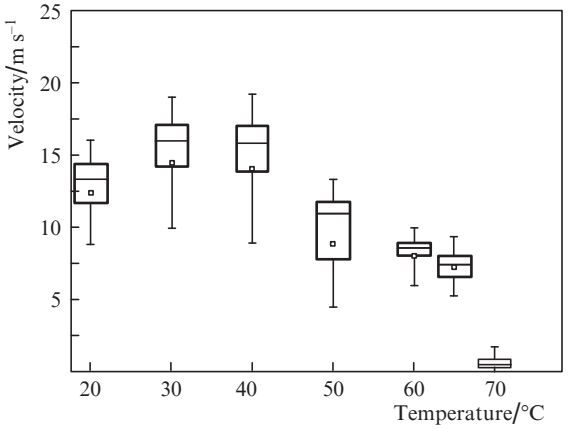

Figure 4. Typical temperature dependence of the velocity of the elastic wave propagation, measured by phase-sensitive OCT.

For a quantitative calculation of the elastic modulus E by formula (4) the cartilage tissue density was taken equal to ρ = 1600 kg m^{-3}. The calculation results for the three test samples were averaged. The resulting temperature dependence of Young's modulus is shown in Fig 5. At room temperature E = 1 MPa, which within the error coincides with the literature data for Young's modulus of cartilage tissue in the elastic compression [25]. The modulus E increases when the temperature rises up to 40 °C, then, in the temperature range from 40 to 70 °C, the tissue elasticity significantly reduces. Qualitatively similar results were obtained in [12] by laser heating of cartilage tissue. In the experiment described, the cartilage plate was compressed and the magnitude of the internal stress was measured in the heating process. It is shown that the internal stress increases, reaches a plateau near 65 °C, and then decreases with increasing temperature. It was assumed that the transient increase in the internal stress during the irradiation may be due to the thermal expansion of water in the heated tissue volume. Internal stress and Young's modulus characterise the elastic properties of matter, and these parameters have qualitatively similar dynamics when heated. It was revealed in the OCT experiments that the characteristic temperature of the beginning of a decrease in Young's modulus lies between 40 and 50 °C.

Figure 5 also shows the dependence of Young's modulus of cartilage tissue on temperature in the range from 20 to 65 °C as measured with a mechanical elastomeric system. The measurements were performed on three samples; the graph represents the average values. It can be seen that the data obtained by the estimates of the propagation rate of elastic waves using phase-sensitive OCT and the data obtained by a mechanical elastomeric system are in agreement at the temperatures ranging from 20 to 40 °C. Thus, we can conclude that the measurement of Young's modulus by phase-sensitive OCT is quite correct. Slight discrepancies may be caused by the differences in the samples (taken from one and the same cartilage tissue) used in the measurements by both methods. (One and the same sample cannot be used in both measurements since the slow heating leads to irreversible changes in cartilage tissue [11].) At higher temperatures, both methods give qualitatively similar results: Young's modulus decreases with increasing temperature in the range from 40 to 70 °C. Nevertheless, there are quantitative differences in the data measured by OCT and the mechanical elastomeric system. Previous studies [20] have shown that the OCT measurements have significantly lesser accuracy than in case of the mechanical elastomeric system; the latter are presented in this paper as a standard for determining the validity of the results.

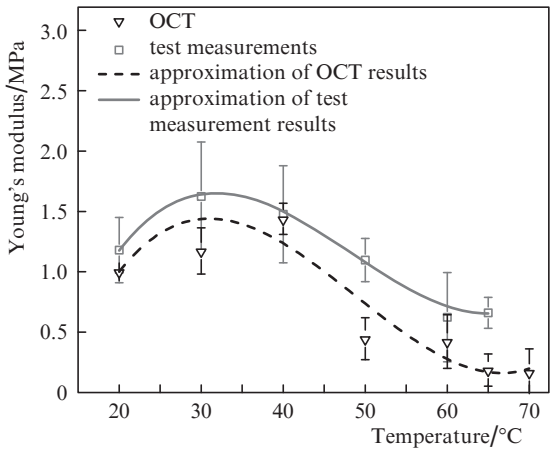

Figure 5. Temperature dependence of Young's modulus of cartilage tissue, obtained by OCT and the test method of mechanical compression. The measurements were carried out for three different samples of cartilage tissue; the averaged values, variance, and the results of fitting the data using the third-order polynomials are shown; the determination coefficient is 0.82 for OCT and 0.98 for the method of mechanical compression.

Papers [26, 27] describe various mechanisms of stress relaxation by laser heating of the cartilage tissue: transition of water from the bound to free state, local depolymerisation of proteoglycans, formation of the microcrystals of salts, polygonisation (the alignment of chondrons which minimises the elastic energy of the system) and also formation of pores in

the cartilage matrix (in which the elastic energy of the cartilage matrix transforms into the surface energy of the pores under formation). In different situations, the manifestation and relative contribution of these mechanisms depend on the tissue structure and external conditions (applied pressure, temperature, exposure time). Some of the processes that occur in this case are reversible (for example, the transition of water from the bound to free state, formation of crystals of the soluble salts), and therefore the cartilage shape subsequently returns to its original state, whilst some mechanisms lead to a stable change in the form that allows the use of the process of laser correction of the shape in medical practice. It is important that after laser irradiation, when the cartilage is cooled to the initial temperature, its elastic and functional properties turn out almost completely restored. However, this does not occur if the time of tissue exposure to high temperature is large enough for the development of the denaturation process of the collagenic subsystem, which, from the medical viewpoint, represents an undesired effect of laser irradiation.

The studies carried out in this paper demonstrate the effectiveness of the OCT method for noninvasive monitoring of the velocity of sound waves and elastic properties of cartilage tissue. However, the results obtained (with the use of a slow, long-term heating of the cartilage in a water bath) are conditioned by the simultaneous occurrence of different mechanisms of stress relaxation (including unwanted denaturation of tissue) and cannot be directly extended to the case of relatively fast (short-time) laser exposure.

The dependence of the velocity of sound in the cartilage on the temperature (Fig. 4) is in good agreement with the temperature dependence of the intensity of the radiation transmitted through the cartilage sample [28], which has been explained by the scattering of light on gas bubbles. The bubbles of gases (oxygen, carbon dioxide) arise in the intertissue fluid during heating due to the temperature dependence of solubility of gases [29, 30] and may cause changes in the velocity of sound. The presence of gas bubbles in a heated cartilage may also explain a noticeable difference in the values of Young's modulus of cartilage tissue obtained at the temperatures above 50 °C by the OCT method and the test method of mechanical compression (Fig. 5). Young's modulus was calculated according to the formula [26] by using the value of the velocity of sound, which depends on the presence of gas bubbles and pores. Since the relative volume of gas bubbles and pores in the cartilage is not too large, their direct effect on the mechanical properties of tissue should be considerably less.

Not all gas bubbles and pores resulting from laser heating are stable in time. After cooling cartilage tissue, a significant portion of gas bubbles and pores collapses, which leads to reversibility of optical and elastic properties of the cartilage [28]. Nevertheless, the results obtained in this work demonstrate a sufficiently high sensitivity of the OCT method and the prospects of its use for noninvasive monitoring of the stress relaxation processes in laser correction of the cartilage.

4. Conclusions

The possibility of using phase-sensitive OCT for monitoring the changes in the elastic properties of cartilage tissue during the heating process is shown. The method of phase-sensitive OCT was used to measure the group velocity of propagation of elastic waves excited by means of a system supplying concentrated air pulses. The value of the group velocity of elastic waves was used to evaluate Young's modulus of cartilage tissue. The resulting temperature dependence of Young's modulus is in agreement with the results of measurements carried out with a mechanical elastomeric system, which indicates the correctness of the method proposed. This noninvasive experimental technique for measuring the mechanical properties of biological tissues is suitable for the use *in vivo*. The development of the method described has an application potential in the clinical conditions for monitoring during the laser reconstruction of the cartilage tissues.

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