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Effect of repetitive laser pulses on the electrical conductivity of intervertebral disc tissue

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***Abstract*. The thermomechanical effect of 1.56-μm fibre laser pulses on intervertebral disc cartilage has been studied using ac conductivity measurements with coaxial electrodes integrated with an optical fibre for laser radiation delivery to the tissue. The observed time dependences of tissue conductivity can be interpreted in terms of hydraulic effects and thermomechanical changes in tissue structure. The laser-induced changes in the electrical parameters of the tissue are shown to correlate with the structural changes, which were visualised using shadowgraph imaging. Local ac conductivity measurements in the bulk of tissue can be used to develop a diagnostic/monitoring system for laser regeneration of intervertebral discs.**

Keywords: *electrical conductivity, biological tissues, cartilage, intervertebral discs, laser.*

1. Introduction

In recent years, a number of medical technologies have emerged that utilise low-invasive physical techniques to treat visceral tissue. One such technique is laser regeneration of intervertebral disc (IVD) tissue [1 – 5]. To ensure effective and safe treatment, it is appropriate to monitor the physical parameters of a tissue that influence its response to the treatment and the laser irradiation time [6].

The mechanical properties of IVDs are determined by the structure and physicochemical properties of the IVD tissues: nucleus pulposus, annulus fibrosus and cartilaginous endplates. Being highly hydrated charged polymer composites, these tissues possess well-defined mechanoelectrical properties [7 – 11]. In previous work, tissue electrical conductivity was studied in relation to the fixed charge on cartilage proteoglycan aggregates [10], the charge of the solutions that constitute the interstitial fluid [9] and charge displacement in the tissue matrix [12]. Attempts were made to assess the composition and state of cartilaginous tissue using streaming potential measurements [13 – 15]. In particular, Buschmann and Legare [14] measured (within a few minutes) the potential distribution associated with intra-articular fluid displacement using an Arthro-BST^{TM} probe (BioSyntech Inc., Canada) in the form of a 4×4 mm 36-microelectrode array. The measurements reported in [14, 16] were made at dc and were unstable because of electrode polarisation. Moreover, such measurements are difficult to combine with a laser radiation delivery system. Electromechanical spectroscopy of cartilage [13] allows one to analyse only the surface conductivity of the tissue. In this paper, we describe local ac conductivity measurements in the bulk of IVD tissue exposed to laser radiation.

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2. Materials and methods

As IVD tissue specimens for conductivity measurements, we used slices of fresh bovine and ovine IVD tissues of two adjacent caudal vertebrae obtained at an abattoir. The specimens were kept in physiological solution at a temperature of 2 – 4 °C for no longer than 48 h. Irradiation was performed with repetitive Er-doped fibre laser pulses ($\lambda = 1.56$ μm) using a 400-μm-diameter optical fibre.

The fibre was passed through a puncture needle, with electrodes placed at the needle tip (Fig. 1). The electrodes were situated in the immediate neighbourhood of the laser exposure region, whose size (~ 1 mm) was determined by the radiation absorption depth in the tissue. The average irradiation power was varied from 0.5 to 1.5 W, and the

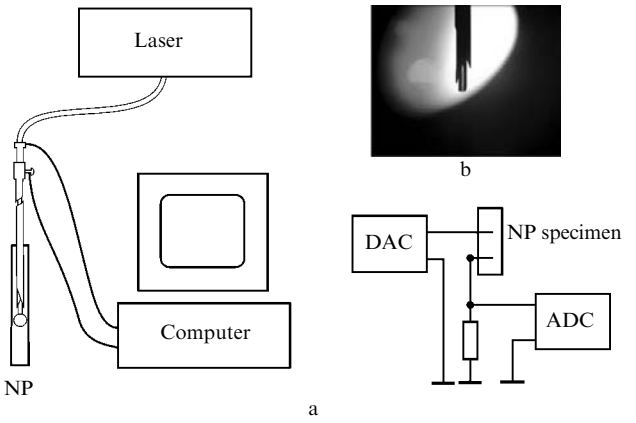


Figure 1. (a) Schematic of the experimental setup for measuring the conductivity of IVD nucleus pulposus (NP) tissue. (b) Puncture needle fitted with an optical fibre for tissue irradiation and with electrodes for conductivity measurements.

pulse width and repetition rate were adjusted so as to maximise the thermomechanical effect observed visually as tissue displacement under laser irradiation. The electrical conductivity of the IVD nucleus pulposus tissue of animals was measured on selected specimens using purpose-designed electrodes, a puncture needle and a computer-based data acquisition and processing system (Fig. 1). The needle tip was inserted into the specimen so that the electrodes were fully immersed in the tissue. The measurements were made at ac, using a National Instruments MIO-16E Series board. The sinusoidal voltage was generated by a digital-to-analogue converter (DAC) of the NI MIO-16E. The current through the tissue specimen was measured using an analogue-to-digital converter (ADC). The electrical circuit for measuring the ac voltage proportional to the current through the specimen is shown schematically in Fig. 1a. To program the NI DAC board and create a user interface, we employed a C++ compiler in the Visual C++ 6.0 environment. The program operated in a single-frequency or multifrequency regime. To optimise the conductivity signal, dependent on the frequency of the sinusoidal signal, the transfer ratio was measured as a function of the amplitude and frequency of the applied voltage.

When measuring the conductivity of the tissue under laser irradiation, we monitored structural changes in the irradiated zone of the tissue. To correlate the conductivity results with the structural changes in the tissue being irradiated, the tissue was filmed by a SONY DCR-TRV 40E digital video camera concurrently with the conductivity measurements. The images were stored via an IEEE 1394 interface on a computer. To improve the tissue image contrast, the system was housed in light-tight enclosure (*10*) (Fig. 2). The light source was red light-emitting diode (LED) (*8*). To synchronise the video recording process and electrical measurements, the LED was turned on only during the measurements. A frame grabber program was developed with a full-colour frame transfer rate of 25 fps for a resolution of 768×576 pixels.

The needle fitted with the conductivity sensor was tested by measuring *in vitro* the conductivity of a liquid, conductive gel and nucleus pulposus tissue harvested from a calf IVD. To optimise the amplitude frequency response of the tissue conductivity, the measurements were made at frequencies from 10 Hz to 100 kHz. The frequency-dependent conductivity data for the above materials showed that the impedance was a hyperbolic function of frequency and was determined primarily by the capacitance of the electrodes near the needle tip.

We determined the frequency at which laser exposure produced the largest changes in conductivity. Accordingly, the time variation of tissue conductivity in response to repetitive laser pulses was studied at an ac frequency $f = 100$ Hz. Er laser irradiation was performed in continuous, single-pulse and repetitive-pulse regimes with a preset number of pulses in the train and a fixed time separation. Concurrently, we measured the current response to an applied sinusoidal voltage $U = 1$ V and determined the conductivity value.

We examined the effect of cw and pulsed laser exposures on the conductivity signal and tissue structure near the electrode sensor. Concurrently, the tissue near the sensor was filmed. The time variation of tissue conductivity was studied as a function of the duration and number of pulses, their repetition rate and laser exposure time. The pulse duration was varied from 100 ms to 2 s, and the pulse repetition rate was 10 to 0.3 Hz.

The temperature in the irradiated zone of the tissue was monitored with thermocouple (*6*) situated at the fibre end (Fig. 2).

3. Experimental results

Analysis of the conductivity in the IVD tissue as a function of the ac amplitude and frequency indicated that the conductivity response had a low-frequency reactive component sensitive to laser-induced changes in the tissue.

When the specimens were exposed to five 1-s laser pulses at a repetition rate of 0.5 Hz, the tissue conductivity increased almost in proportion to the number of pulses [Fig. 3, curve (*1*)], but 10 s after the exposure the conductivity returned to its initial level. The conductivity curve measured during re-exposure of the tissue at the same

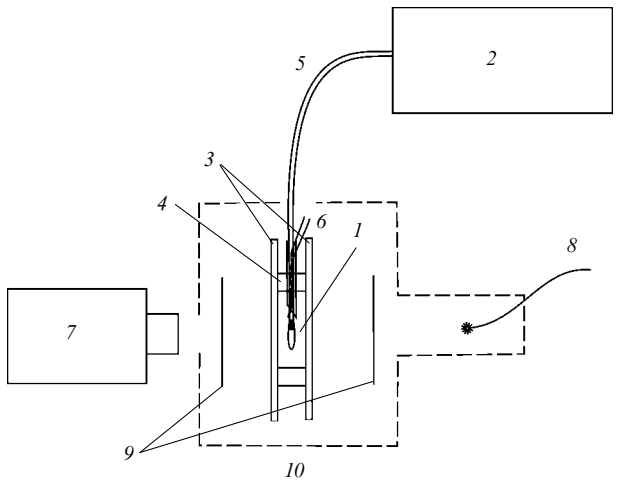


Figure 2. Schematic diagram of the video recording of the tissue during laser exposure: (*1*) biological tissue specimen; (*2*) laser; (*3*) transparent glass plates; (*4*) slide; (*5*) optical fibre with a resistance sensor; (*6*) thermocouple; (*7*) digital video camera; (*8*) LED; (*9*) lenses; (*10*) light-tight enclosure.

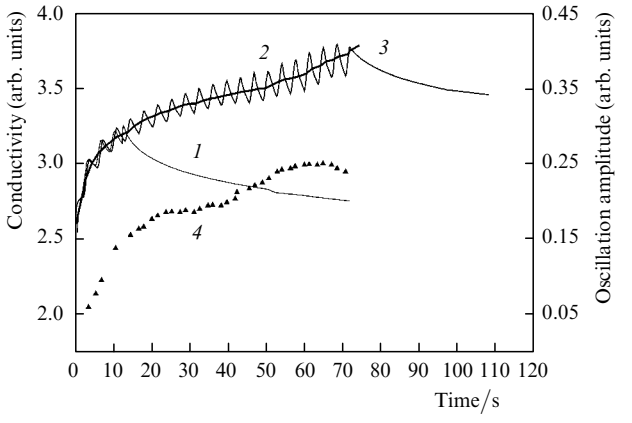


Figure 3. Variation in the conductivity of the IVD nucleus pulposus tissue (*1*) in response to five laser pulses and thereafter, and (*2*) during prolonged exposure to 1-s laser pulses at a repetition rate of 0.3 Hz (average irradiation power, 1.2 W); (*3*) average tissue conductivity; (*4*) conductivity oscillation amplitude.

point first duplicated curve (*1*). After 30 s of irradiation, the rise in conductivity became more gradual, but after 50 s the slope began to increase again [Fig. 3, curve (*2*)]. The conductivity oscillation amplitude [curve (*4*)] increased during the first 20 s and then stabilised, whereas the average conductivity continued to grow [curve (*3*)]. After exposure for 75 s, the tissue conductivity did not return to its initial level [Fig. 3, curve (*2*)]. The increase in average conductivity amounted to 30 % of its initial level after the first 10 s of laser exposure and then slowed down. After 50 s of irradiation, the average conductivity began to rise at an increasing rate until the end of the exposure, where the increase in conductivity amounted to $\sim$40 % of its initial value.

The observed changes in conductivity were qualitatively reproducible in measurements on different IVDs (seven discs) of the same animal. Irradiation with three trains of 1-s pulses at a repetition rate of 0.5 Hz and average power of 1.5 W increased their conductivity by 20 % – 40 %.

Structural examination of the irradiated region showed that the tissue was periodically displaced from the heated region with the pulse repetition frequency. The tissue temperature also increased periodically, with a maximum value within $\sim$50 – 55 °C. In the fibre – tissue contact region, a cavity was formed, filled with the interstitial fluid (Fig. 4b, light area around the fibre end).

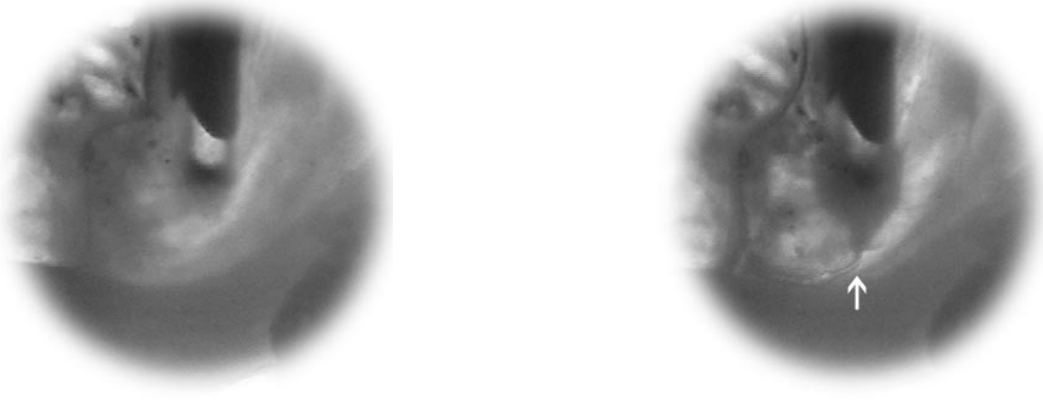
As the number of laser pulses increases, the density and dimensions of the shadowgraph picture increase to the point of rupture of the cavity produced in the tissue near the fibre end, when a superheated tissue fluid begins to exit at a high speed through the hole in the cavity. The fluid jet contains many bubbles (Fig. 5b). Measurement of their velocity by PIV image processing [17] showed that the fluid velocity was $\sim$10 cm s^{-1}.

4. Discussion

The conductivity data for IVD tissues exposed to repetitive laser pulses show that irradiation may produce both reversible and irreversible changes in tissue conductivity, depending on the exposure time [Fig. 3, curves (*1*, *2*)]. Irradiation for a short time causes the tissue to displace from the radiation absorption region to the cold zone. We believe that the displacement is due to the thermal expansion of the interstitial fluid in the cartilaginous matrix. In the case of a limited hydraulic conductivity of the matrix, this may markedly raise the intradiscal pressure. After laser exposure, the pressure drops and the tissue reverts back to its original state. During long-term exposure to laser pulses, the cyclic displacement of the tissue with an increasing amplitude continues until the intradiscal pressure of the interstitial fluid is balanced by the tension forces in

a b

Figure 4. Structure of the IVD nucleus pulposus tissue (a) before and (b) after irradiation. The arrows mark the fibre end. The shadowgraph images were obtained by probing the tissue with red light.



a b

Figure 5. Images of a cavity in the IVD nucleus pulposus tissue (a) before and (b) after rupture. At the fibre end, one can see a tissue fluid jet containing bubbles (the rupture point is marked by an arrow).

the matrix [Fig. 3, curve (*4*)]. Further heating of the tissue may lead to collagen denaturation and then to tissue coagulation, resulting in the formation of a coagulation cavity near the fibre end (Fig. 4b). This in turn may reduce the hydraulic conductivity of the tissue, raise the pressure and lead to rupture of the cavity (Fig. 5b).

Analysis of the laser-induced structural changes demonstrates that the tissue becomes denser outside the exposed region and becomes less dense within it, as evidenced by the changes in the optical density of the tissue in the shadowgraph image (Fig. 4). Thermocouple measurements show that the tissue temperature does not exceed the collagen denaturation temperature outside the laser beam and considerably exceeds it in the region under irradiation, which probably leads to changes in the collagen, in particular, in the density of the tissue and the permeability of the fibre network. Long-term laser exposure stabilises this tissue density distribution, leading to irreversible changes in the structure and electrical properties of the tissue.

The tissue displacement relative to the interelectrode space leads to redistribution of free ions in the interstitial fluid, increasing their contribution to the tissue conductivity. Moreover, tissue denaturation may cause bound counterions to contribute to the tissue conductivity. All this raises the conductivity in the region under laser irradiation. The cyclic displacement of the tissue (collagen fibres, which have lower conductivity) modulates the current through the interelectrode space, which can be used to probe the mechanical state of the tissue. The current modulation amplitude reflects the amplitude of mechanical oscillations [Fig. 3, curve (*4*)], and the average conductivity reflects the concentration of conduction ions in the coagulation cavity. Note that heating of the fluid in the closed coagulation cavity near the fibre end may lead to rupture of the tissue and release of the heated fluid to the surrounding space. The fluid velocity may be rather high ($\sim$10 cm s^{-1}), leading to bubble formation (cavitation). Rupture of the cavity is undesirable because of the hazard of a thermal influence on the spinal cord tissue situated in the immediate vicinity of the exposed zone. Consequently, conductivity measurements can be used to monitor the state of the tissue in the zone under laser irradiation.

5. Conclusions

The present results demonstrate that the electrical conductivity of IVD tissue increases with the number of laser pulses and oscillates at the pulse repetition frequency. The oscillation amplitude first increases and then stabilises. Concurrently, the rise in average conductivity slows down sharply. Further increasing the exposure time leads to a steep rise in average conductivity with no changes in oscillation amplitude. Laser irradiation alters the structure of the nucleus pulposus tissue in the exposed region. The structural changes correlate with the changes in tissue conductivity. Therefore, local ac conductivity measurements in the bulk of tissue can be used to develop a diagnostic/monitoring system for laser regeneration of IVDs.

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