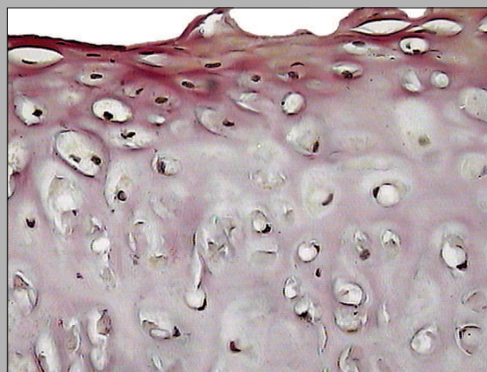


Abstract: Demonstration of laser-induced stress relaxation in cartilage in 1992 led to the development of a novel laser surgical procedure in otolaryngology for the non-ablative reshaping of cartilage. Follow-up studies found that non-destructive laser irradiation may activate regeneration processes in cartilaginous tissue. Ongoing studies seek to characterize the physical, chemical and biological processes and mechanisms involved in the reshaping and regeneration of deformed and diseased cartilage under moderate laser heating. A theoretical model is developed considering laser-induced stress relaxation in cartilage as a process of micropore formation. Results obtained provide scientific and engineering data for development of novel laser surgical procedures for correction of the nasal septum and treatment of spine disc cartilage diseases. This review is aimed to present state of art and recent results in laser – induced reshaping and regeneration of cartilage.



Histological cross-section of porcine nasal septal cartilage after laser reshaping using $1.56 \mu\text{m}$ laser radiation with power of 1.5 W, spot diameter 2 mm, exposure time of 5 s. Stained with Hematoxylin and Eosin, $\times 400$

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Laser reshaping and regeneration of cartilage

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1. Introduction. The problems. Phenomenon of laser reshaping and regeneration

Many human diseases are related to deformed or degenerated cartilage. Twenty-five percent of the population suffers from a bent nasal septum. Over time, back pain will affect 80 – 90% of the population. Direct and indirect yearly medical expenses from degenerated cartilage in the USA are more than 50 billion.

Cartilage is an ancient biological tissue having existed in some of the earliest organisms. Cartilage acts as a shock-absorber, mechanically connects, supports and maintains various tissues and organs. In the human em-

bryos – about 40 percent of the body mass is cartilage. During the embryonic development of a living organism, cartilage transforms to bone, muscles, and other tissues. Cartilage contains neither nerves nor blood vessels and is an ideal material for transplantation. Because cartilage possesses a high internal stress and exhibits shape memorization, realizing permanent cartilage shape change has been difficult. In 1993, we reported results of early experiments to change the shape of cartilage using non-destructive laser irradiation [1].

The idea underlying the procedure is to first mechanically form a desired shape and then to irradiate the area of maximal stress to produce stress relaxation and fix a new

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cartilage shape [2,3]. The initial laboratory demonstration led to laser septochondrocorrection – a surgical procedure for laser correction of deformed cartilage septum in the human nose [4]. In comparison to the highly traumatic conventional surgery to correct septal deviations, laser septochondrocorrection is a painless, bloodless, outpatient procedure that requires about 10 minutes to complete. Moreover, candidate patients for laser septochondrocorrection are not limited by age nor do they suffer from secondary effects.

The effect of non-destructive laser irradiation on the stress distribution in cartilage allows not only changing cartilage shape but also opens the possibility to activate tissue regeneration processes [3,5]. Heterogeneous laser heating under modulated laser irradiation create micro pores and channels that improve water permeability and delivery of nutrients to chondrocytes. Pulsatile and repetitive mechanical stress of a certain amplitude activates chondrocyte proliferation and production of a new cartilage matrix. As a result, the controlled application of laser radiation on the microstructure and stress distribution in tissue leads to generation of hyaline cartilage [3].

These results suggest that a new approach for laser reconstruction of cartilage might be developed that is very promising for orthopedics and spine surgery [6]. Over the last ten years a number of research groups in the United States, Greece, United Kingdom, France, Italy, Egypt, and Spain have begun research studies in non-ablative laser treatment of cartilage. This review is aimed to present state of art in laser – induced reshaping and regeneration of cartilage.

2. Mechanical processes: Stress relaxation in Cartilage

Cartilage belongs to the family of hard biological tissues due to high internal stress and the property of “stress memorization”. Cartilage consists of water (70-80 percent), a collagen fiber network and proteoglycans [6]. The proteoglycans have a multitude of negatively charged side groups that produce electrostatic repulsion. When a cartilage slab is bent, distance between negatively charged groups decreases, and mechanical stress returns cartilage to its initial shape. If stress relaxation occurs, internal forces causing cartilage to return to the initial shape disappear, and a new and stable shape can be realized. Laser-induced stress relaxation is connected with a short-lived decrease in cartilage mechanical properties, since the steady state Young’s modulus does not change significantly after laser reshaping from initial values [3,7]. The stress-strain relationship recorded from human nasal septal cartilage has been presented in Fig. 1.

Testing of cartilage immediately after laser treatment shows three characteristic deformation regions. First, a region of small strain deformation ($\epsilon \leq 0.05$) corresponds to a two-fold decrease of Young’s modulus compared to ini-

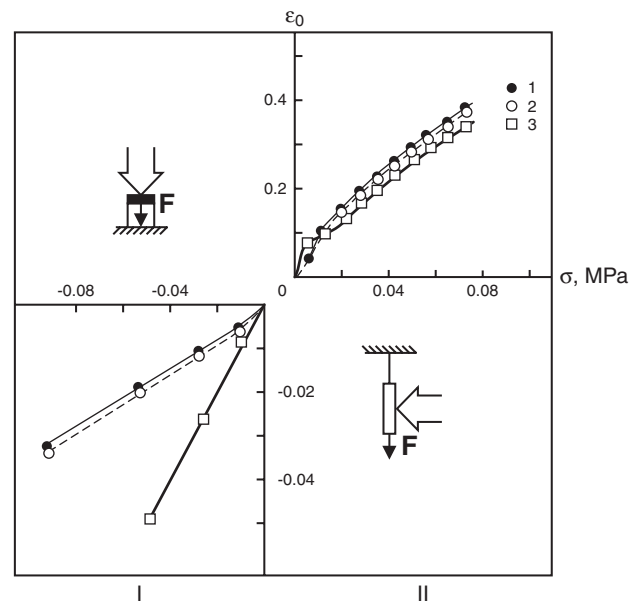


Figure 1 Stress-strain dependencies for cartilage slab irradiated. 1 – Initial cartilage; 2 – Irradiated sample after 10 minutes soak in buffer solution; 3 – Cartilage sample immediately after irradiation

tial values. Second, a region of expansion with ϵ increasing from 0.05 to 0.4 shows a negligible change of Young’s modulus. Third, in compression, Young’s modulus is an order of magnitude higher than that for expansion. During laser irradiation, Young’s modulus decreases by approximately two times. The mechanical properties (e.g., Young’s modulus) of the irradiated specimens return to initial (non-irradiated) values after immersing the laser irradiated cartilage specimen for ten minutes in physiologic saline.

Two regions of “low” and “high” rigidity have been studied for cartilage [8–11]. The “low rigidity region” [10] represents relatively small deformations ($\epsilon < 0.1$) and is governed by the proteoglycan subsystem of the cartilage matrix, while the “high rigidity region” represents high deformation ($\epsilon > 0.1$) and is governed by tension in the collagen network.

Our study of time variation of cartilage deformation in the course of laser irradiation under fixed mechanical load ($\sigma = 0.02$ MPa) indicated a transitional behavior followed by a steady-state [7]. Laser-induced strain alterations result in small deformations ($\epsilon < 0.1$) representing the low rigidity region of cartilage. Changes in cartilage in the high rigidity ($\epsilon > 0.1$) region did not change in the course of laser irradiation [6]. Based on these results, we concluded that the proteoglycan subsystem in the cartilage matrix is primarily responsible for laser-induced stress relaxation in cartilage [6].

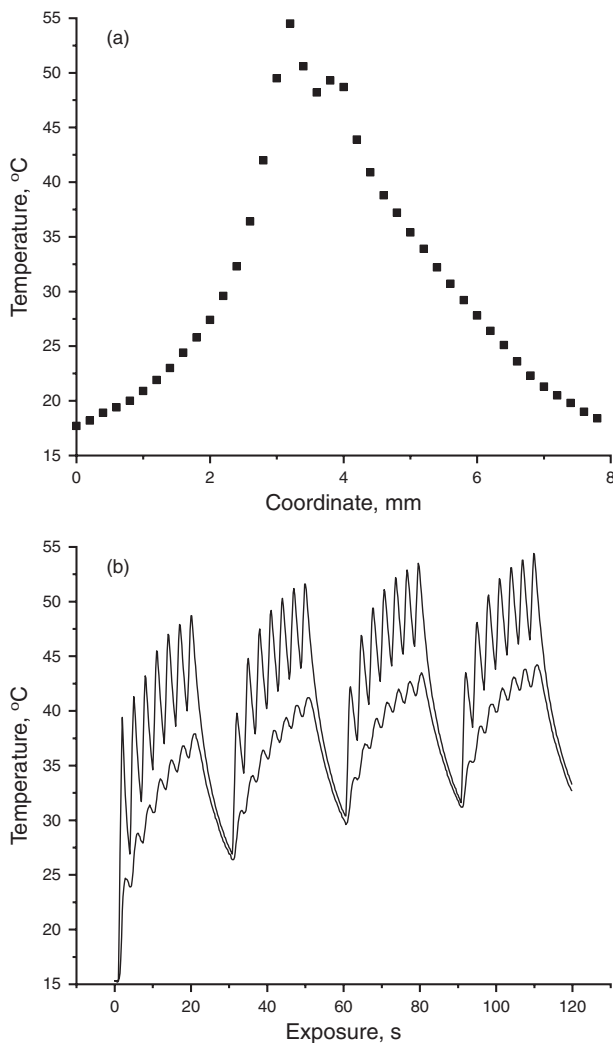


Figure 2 Temperature of a nucleus pulposus of rabbit spine disc irradiated by $1.56 \mu\text{m}$ laser radiation (power 1 W, pulse duration 2 s, break between pulses 1 s; the 10 s pause followed every set of six laser pulses after which laser exposure was continued): (a) – spatial distribution of temperature at the irradiated surface for 110 s of irradiation; (b) – temperature dynamics for two points: Upper curve refers to the central region of the laser spot, lower curve refers to a point 1 mm from center of laser irradiation

Using optoacoustics, the viscoelastic properties of laser-irradiated cartilage have been studied utilizing repetitive pulsed-laser excitation of mechanical oscillations [6,12]. The effects of laser power, pulse duration, repetition rate, and irradiation time on the shape of the optoacoustic signal have been analyzed. Optoacoustic response of cartilage to repetitive pulse-laser excitation depends on the thickness and mechanical characteristics of the cartilage specimen (elastic modulus, hydraulic permeability) and correlates with temporary tissue softening observed during laser-induced stress relaxation [13].

3. Thermal processes under laser radiation

Laser-induced stress relaxation in cartilage is based on temperature dynamics in the tissue. The observed correlation between stress and temperature in cartilage during laser irradiation is longstanding [2,3,14–16]. Permanent stability of the new cartilage shape following laser irradiation is achieved only when a critical temperature $T_c = 70 \pm 5^\circ\text{C}$ is reached. For lower temperatures, shape change is temporary and initial shape of cartilage is restored after a few tens of minutes.

Actually, the temperature can be non-uniform in a laser-irradiated cartilage specimen. At least half of the cartilage thickness must be heated over T_c , for effective stress relaxation. Real spatial and temporal temperature distribution can be determined on the basis of a theoretical model or from experimental measurements of cartilage temperature during laser irradiation.

Temperature in the bulk of septal cartilage was measured with needle-shaped thermocouples ($30 \mu\text{m}$ in diameter) inserted into a small incision (depth of 0.5 mm) in the posterior side of a septal cartilage specimen [15]. Invasive methods of temperature monitoring are more traumatic for the patient during laser surgical procedures. In comparison, surface temperature can be measured more easily, for example with an infrared radiometer. An infrared focal plane array camera ($3 - 5 \mu\text{m}$ sensitivity) was used to measure spatial and temporal distributions of surface temperature in cartilage specimens over the course of laser radiation.

Fig. 2 presents a measured temperature field in a rabbit intervertebral disc during periodic pulsed laser irradiation. Temperatures were measured by a Thermovision camera CEDIP JADE LWIR. Amplitude of temperature oscillations (Fig. 2b) decrease rapidly with increasing distance from the center of the laser irradiation spot.

Calculation of the laser-induced temperature field requires solving the heat conduction problem with appropriate boundary conditions accounting for space-time dependence of optical fluence and thermal properties [2,17]. Numerical implementation of our theoretical model allows calculating the temperature field in nasal septal cartilage covered by mucosa. Since $1.56 \mu\text{m}$ laser radiation is absorbed by cartilaginous water, and as water distribution in the irradiated cartilage specimen is inhomogeneous due to water evaporation and/or water movement [6,18], maximum temperature is shifted from the surface to deeper layers [18].

Non-uniform irradiation of tissues with pulsed lasers leads to a non-uniform thermo-elastic expansion and, accordingly, to tension and deformation. Stress in cartilage increases in the course of laser heating. When stress reaches a plastic threshold, plastic deformation occurs, and stress decreases. Subsequent cooling of the zone where laser-induced stress relaxation occurs will result in residual stress in this area. In the first approximation, stress in cartilage during laser irradiation can be calculated using thermo-elastic stress theory.

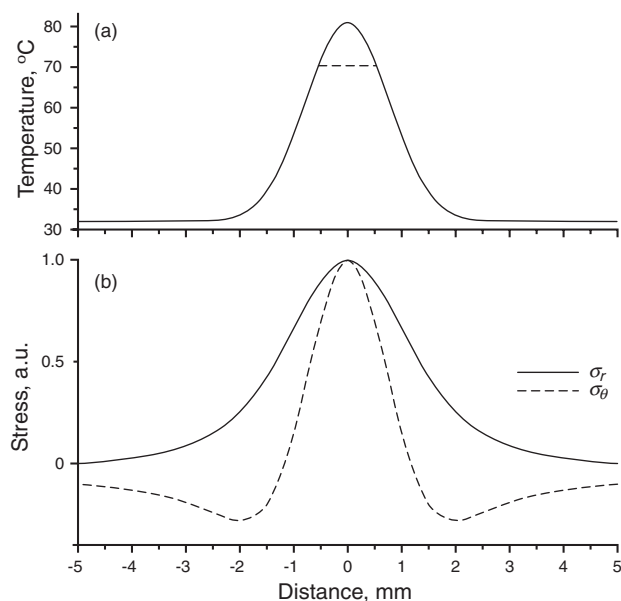


Figure 3 Spatial distributions of (a) temperature and (b) thermo-elastic stress in laser-irradiated cartilage. The radial (σ_r) and angular (σ_θ) stress are in arbitrary units. The temperature distribution has been calculated for the fourth second of irradiation for laser power of 0.7 W. The laser source had Gauss distribution with the characteristic radius of 1 mm. The dashed line at 70°C on the temperature graph corresponds to the beginning of stress relaxation

Computed space distribution of temperature and thermo-elastic stress in cartilage is shown on the Fig. 3.

Assuming that plastic deformation occurs at some threshold value of stress, we can estimate conditions for plastic deformation resulting in stress relaxation in cartilage. Alternatively, the conditions of plastic deformation can be defined by von Mises criterion [19]: $\sigma_\theta - \sigma_r = \sigma_S$, where σ_S is a plasticity threshold. Taking 70°C as a threshold temperature for laser-induced stress relaxation of cartilage [3], the von Mises criterion gives σ_S as 12 MPa. The last value conforms to models of polymer plasticity [20] which give $\sigma_S \sim 7$ MPa. The above calculation indicates that a simple consideration using thermo-elastic theory taking into account von Mises criterion, can give reasonable estimates for the threshold of laser-induced stress relaxation.

4. Phase transformations and chemical processes during laser irradiation of cartilage

Phase transformations and chemical processes in laser treated cartilage may progress through a number of stages. First stage of laser modification of cartilage structure and mechanical properties is the transformation of water state and structure [3,6] due to alterations of water-water and water-biopolymer interactions [21], in particular, the

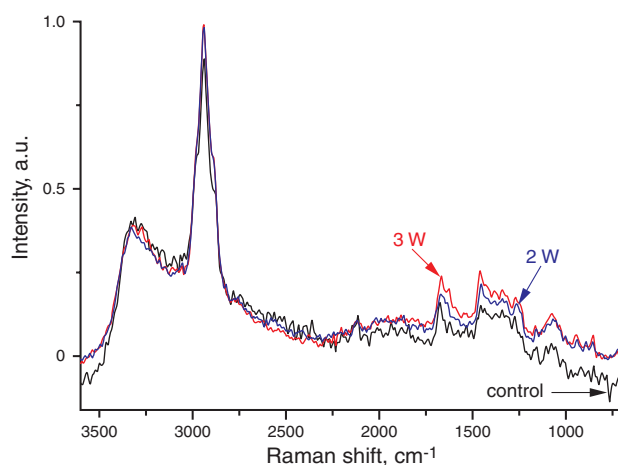


Figure 4 (online color at www.lphys.org) Raman spectra of bovine nasal septa cartilage intact and after 1.56 μm laser treatment of 2 and 3 W in power (respectively 8 and 12 W/cm^2)

bound-to-free phase transformation of cartilaginous water [2,22]. The first stage may be followed by various processes including alterations in collagen and proteoglycan subsystems, local mineralization, local melting, and creation of micropores in the cartilage matrix. One of the first responses of cartilage to laser heating is water evaporation and tissue dehydration. Water evaporation during irradiation was studied theoretically [18] and experimentally by pulsed photothermal radiometry [23] and FTIR technique [24]. Youn et al. demonstrated that dehydration is a principal source for changes in birefringence in cartilage, observed by PS-OCT technique [21].

Decreased water content in cartilage following laser irradiation can result in precipitation of salts. Using atomic force microscopy, Sobol et al. [25] observed formation of small sub-micron crystals in the laser irradiated zone of cartilage. Using Raman microspectrometry, Heger et al. [26] identified intracellular calcium sulfate deposits and extracellular calcium phosphate (apatite) crystals in laser-reshaped rabbit auricular cartilage.

Cartilage may be viewed as a porous and permeable composite made up of a water-saturated organic solid matrix [3,6]. Primary components of the solid matrix are water, type II collagen fibers, proteoglycans and their aggregates with hyaluronan. These macromolecules are characterized by primary, secondary, tertiary structures and form the supramolecular organization and framework of the extra-cellular matrix.

Primary structure of macromolecules does not change during non ablative infrared laser irradiation of connective tissue. Raman spectroscopy study of laser irradiated samples of nasal cartilage has not detected formation of any new bands (Fig. 4) [27]. Electron Spin Resonance investigation of cartilage and its components (collagen and glycosaminoglycans) during IR laser irradiation (up to 40 W/cm^2) did not reveal any radical generation [6]. These

observations suggest that no covalent bonds rupture as a result of laser reshaping of cartilage.

Proteoglycans in cartilage act as thermal stabilizers and the triple helix (the secondary structure) of type II collagen in nasal septal cartilage is stable up to 100°C [28–30]. Only partial collagen denaturation under laser irradiation was detected by calorimetric, histological and enzyme digestion methods [31]. These studies indicate the highest possible extent of collagen denaturation is 15.5%. The fraction of degraded collagen in nasal septal cartilage after reshaping does not exceed that in non-irradiated samples. These observations are consistent with the absence of direct histological observation of laser-induced alterations in cartilage structure in the first studies of laser reshaping of cartilage [6,22]. Nevertheless, future examination of cartilage matrix structure revealed some ultra structure alterations resulting from laser irradiation. Light microscopy examination of picosirius red stained sections indicates that matrix de-condensation and fibrillation occur in laser treated articular cartilage samples [32]. Collagen fiber modification is also confirmed indirectly. For example, the monolayer water content, decreases after laser irradiation [30] and the enthalpy of collagen denaturation decreases [28]. These data are consistent local partial fusion of collagen fibers.

Some rarefaction of collagen structure due to laser-induced increase in diameter of collagen fibrils was observed [22]. Later Hayashi et al. [33] using transmission electron microscopy reported similar findings (increased fibril cross-section diameter) in their study evaluating the effect of Ho:YAG laser irradiation at non-ablative levels on the ultrastructural properties of the joint capsular collagen. Non ablative laser treatment causes disruption of regular fibril organization at temperatures above 70°C for laser treatment durations longer than 10 s [28,34]. Examination of cartilage ultra structure using Atomic Force Microscopy (AFM) has shown that clinically relevant regimes of laser reshaping of cartilage do not lead to collagen denaturation [6,25]. Unlike hyaline cartilage, type II collagen in the nucleus pulposus (NP) of the intervertebral disc denatures upon heating to 75°C [34] and partial destruction of collagen triple helix structure in NP after laser irradiation was observed.

Changes in the cartilage proteoglycan subsystem can occur due to non-ablative laser irradiation. Size exclusion chromatography/multi-angle light scattering and sedimentation velocity measurements showed that laser irradiation can decrease the molecular mass of glycosaminoglycans [35]. Histochemical analyses demonstrated that destruction of proteoglycans and proteoglycan aggregates occur [34]. The release of proteoglycans destroyed during laser heating and diffusion of macromolecules into the medium suggest depolymerization of PG and their aggregates [34,35]. AFM studies show that laser irradiation of various cartilage types effects porosity by formation of nano-pores [3,6,25]. These nano-pores improve delivery of nutrients to chondrocytes which is required for regeneration and reparative processes in cartilage.

5. Optical processes. Light scattering dynamics

A correlation between alterations in optical properties and stress relaxation during laser irradiation of cartilage was reported in 1996 [15]. A nasal septal cartilage specimen was bent mechanically and the area of maximal stress (the crease) was irradiated with a Holmium YAG laser ($\lambda = 2.1 \mu\text{m}$). The temperature, stress and transmitted light intensity during laser irradiation were monitored simultaneously. Stress relaxation in cartilage began when temperature reached about 70°C while simultaneously transmitted light intensity was at a minimum. Stress relaxation resulted in a stable and permanent shape change. The correlation between light scattering and stress relaxation in cartilage during laser irradiation was studied using integrating spheres [36,37], and using a multichannel fiber-optic collecting the intensity of the probing beam back scattered at various angles from the irradiated area of cartilage sample [6]. Integrated intensity of the back scattered probe beam has an extremum simultaneous with stress relaxation. Measurements of the space distribution of scattered light propagating through cartilage during laser irradiation [38] were used to study dynamics of laser-induced alterations in tissue structure. This approach allowed experimentally establishing denaturation thresholds for cartilage and cornea as a function of laser wavelength [6,38].

Light scattering was used to monitor the dynamics and energy thresholds of laser-induced structural alterations in biopolymers due to irradiation by a free electron laser (FEL) in the infrared (IR) wavelength range from 2.2 to 8.5 μm . Attenuated total reflectance (ATR) Fourier-transform IR (FTIR) spectroscopy is used to examine infrared tissue absorption spectra before and after irradiation. Light scattering by bovine and porcine septal cartilage and cornea samples is measured in real time during FEL irradiation using a 650-nm diode laser and a photodiode array with time resolution of 10 ms. Time dependent light scattering in the septal cartilage and cornea samples are modeled to estimate the approximate values of kinetic parameters for tissue denaturation as functions of laser wavelength and radiant exposure. Results indicate denaturation threshold is slightly lower for cornea than cartilage, and both depend on laser wavelength. An inverse correlation between denaturation thresholds and the absorption spectrum of the tissue is observed at many wavelengths; however, for wavelengths near 3 and 6 μm , the denaturation threshold does not exhibit the inverse correlation, instead being governed by heating kinetics of tissue [38].

5.1. Dynamics of IR light transmission in cartilage in relation to laser irradiation and mechanical loading

Optical properties of nasal septal cartilage at room temperature have been studied in the near IR region using in-

tegrating spheres [6,39,40]. Pulsed photothermal radiometry [23,38,41] has been applied to study the effect of free electron laser radiation on the absorption coefficients of cartilage and cornea. The use of short laser pulses allowed avoiding the effect of water evaporation and movement during laser irradiation. The following laser wavelengths 6.45, 6.35, 6.29, 6.20, 6.09, 6.00, 5.92, and 3.22, 3.15, 3.00, 2.87, 2.82, 2.81 μm have been used in these studies [23]. Inasmuch as these wavelengths are located on the cartilage absorption spectrum approximately symmetrical relative to the peaks of the water absorption bands 6.1 and 2.9 μm , the selected pairs correspond to approximately equal initial absorption.

The absorption coefficients for these wavelengths within the 2.9 and 6.1 μm water absorption bands have been determined for tissue samples irradiated with various laser pulse energies. Similar to absorption by pure water, water incorporated into cartilage and cornea demonstrates a strong dependence of the absorption coefficient on temperature. The absorption coefficient for cartilage in the wavelength range near to the peak of the water absorption 2.9 μm , decreases at temperatures higher than 50°C owing to thermal alterations of water-water and water-biopolymer interactions [23]. When laser light is absorbed primarily by tissue water, water evaporation during laser heating changes bulk distribution of water in the tissue and effects light absorption [41]. Drying of superficial tissue layers leads to a non-uniform water distribution that may explain in-part the difference in measured absorption coefficients using PPTR and IR spectroscopy [41].

The influence of mass transfer of water on the temperature field in cartilaginous tissue under the action of laser irradiation was theoretically studied [42]. Two different mechanisms of mass transfer of water in cartilaginous tissue (molecular-diffusion mechanism and laminar flow) have been considered in solving heat- and mass-transfer equations. The calculations indicate maximum temperature immediately following pulsed laser irradiation is attained inside the sample because of evaporation of water from the air-tissue interface. The influence of different parameters of laser radiation and mass transfer of water on the surface temperature, magnitude of maximum temperature, position of maximum temperature, and characteristic time at which the diffusion-limited relaxation of stresses in the cartilaginous tissue occurs has been analyzed.

The dynamics of laser light transmission through septal cartilage for various powers (P) of radiation at 0.97 and 1.56 μm wavelengths was studied [6]. For 1.56 μm , the transmission does not depend on laser power for low laser power ($P < 1$ W), but increases with time for incident powers greater than 1 W. For 7 s irradiation with $P = 2.2$ W, the transmission increased nearly 2.5 times. The increase of cartilage transparency for this wavelength is mainly due to water evaporation from the irradiated surface and water thermo expansion and movement under a temperature gradient. A second reason for decreased light absorption may be due to a shift of the water absorption band under laser heating [38,43,44]. For 0.97 μm radia-

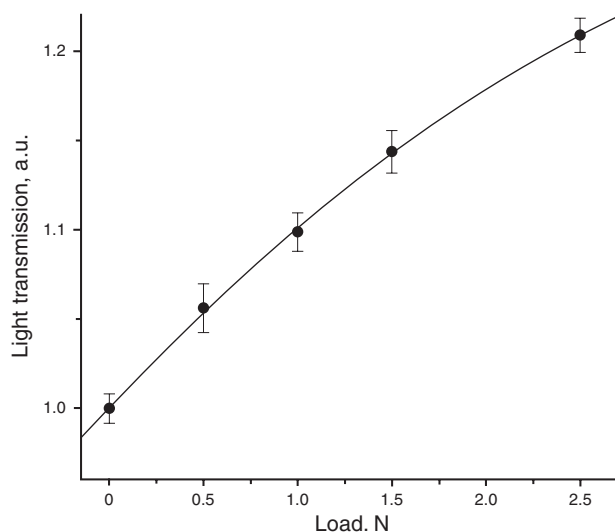


Figure 5 Transmission of 1.56 μm laser light through bovine nasal septum cartilage 1.5 mm in thickness as a function of applied compression load. The load and laser irradiation were applied to the cartilage surface with transparent indenter of 3 mm in diameter

tion, the tissue absorption is not dependent only on water content; alterations in cartilage transparency are different from the case of 1.56 μm , and connected mainly with tissue denaturation [6].

Dynamics of 1.56 μm laser light transmission through cartilage is also studied as a function of mechanical load. The mechanical loading (compression) was applied to a nasal cartilage slab in the direction of laser irradiation. The incident fluence of laser light was small (of 0.5 W/cm^2) to avoid any thermal-induced effects (the temperature increase was less than 1°C).

Mechanical deformation of the cartilage sample was approximately linear with mechanical load increasing from 50 to 250 g. The deformation was 0.22 mm (15 percent) at a load of 250 g. The effect of mechanical load increases transmission (Fig. 5) up to 20 percent. Since 1.56 μm radiation is absorbed mainly by water, the observed alteration in cartilage absorption can be attributed to alterations in water content in the mechanically loaded cartilage.

Thus mechanical loading removes water from superficial layers of cartilage, decreases light absorption in the superficial layer and shifts the maximum of temperature increase to deeper positions in the cartilage.

6. Structural alterations. The optimal laser settings for laser reshaping

Laser-induced structural alterations in cartilage have been studied for different dosimetry including laser wavelength,

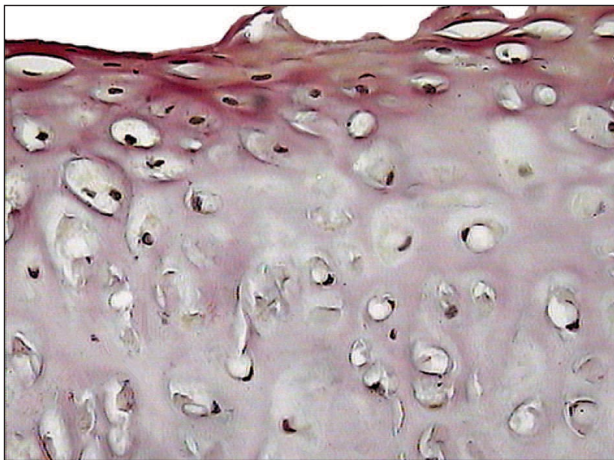


Figure 6 (online color at www.lphys.org) Histological cross-section of porcine nasal septal cartilage after laser reshaping using $1.56\ \mu\text{m}$ laser radiation with power of 1.5 W, spot diameter 2 mm, exposure time of 5 s. Stained with Hematoxylin and Eosin, $\times 400$

fluence, and types of cartilaginous tissues using conventional histological techniques [6,22,32], atomic force microscopy [25,45] and optical methods [38]. Two types of structural alterations have been identified: direct, that appear immediately after laser irradiation and secondary that arise after some time, as a result of tissue regeneration processes [6].

The first studies of laser-induced reshaping processes have shown a laser dosimetry space exists that produces stress relaxation without dramatic alterations in the cartilage matrix [3,6,22,46]. A theoretical model was developed that considers stress relaxation and structural alterations as one process in which the kinetics is governed (limited) by mass transfer processes [18]. In this model, a threshold temperature exists at which portions of the cartilage matrix may move relative to neighboring regions [3,18]. Since the distance of movement is less for stress relaxation than that for denaturation, stress relaxation may occur without tissue denaturation, when the process time is sufficiently short.

Morphological study of porcine nasal septal cartilage irradiated *ex vivo* using CO_2 or Ho:YAG lasers showed pronounced destruction of chondrocytes and collagen fibrils in the perichondrium, while the cartilage matrix showed only weak dystrophic alterations in chondrocytes and some decrease in glycosaminoglycan content [6,22]. The magnitude of these alterations increases with incident laser fluence.

Histological cross-section of nasal septal cartilage after laser reshaping using $1.56\ \mu\text{m}$ laser radiation is presented in Fig. 6. Laser-induced stress relaxation did not change dramatically cartilage matrix structure although weak disruption of the perichondrium and pyknosis to

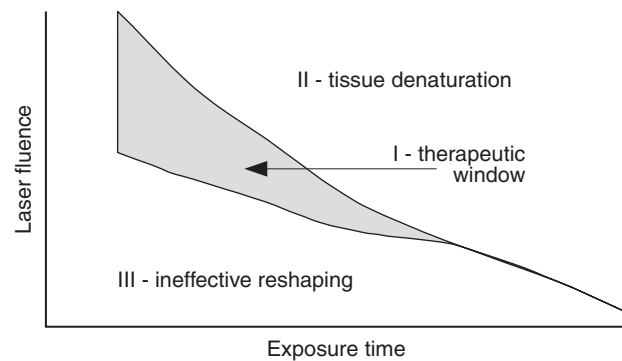


Figure 7 Optimal conditions for laser reshaping of cartilage

some chondrocytes is observed. Experiments indicate that, for $1.56\ \mu\text{m}$ laser radiation, time duration for laser reshaping of human nasal septal cartilage of 1 – 2 mm in thickness is 4 – 10 seconds [6].

Considering possible denaturation to cartilage matrix in the course of laser reshaping of cartilage we must emphasize that both denaturation and laser-induced stress relaxation are the thermal- and time-dependent processes.

Therapeutic window of laser dosimetry for cartilage reshaping has been presented in Fig. 7 (zone I). Lower fluence (zone III) does not ensure stress relaxation. High fluence (zone II) results in undesirable effects, for example tissue denaturation or cell damage. Upper boundary in Fig. 7 represents denaturation of cartilage structure. Since stress relaxation may occur over a shorter time than denaturation, laser reshaping may be accomplished without visible damage or denaturation of tissue structure. A theoretical model of laser-induced structural alterations in biopolymers [18] predicts the existence of a therapeutic window (zone II) allowing stress relaxation without dramatic alterations in tissue structure. Range of laser fluences in the therapeutic window decreases with increasing exposure time. Upper and lower boundaries of the therapeutic window depend on laser wavelength, pulse characteristics, thickness of cartilage specimen, and heterogeneity and water content.

Because chondrocytes are more sensitive to laser treatment than the cartilage matrix [3,6,47], some chondrocytes are damaged and die during laser reshaping. Because cells are sensitive to mechanical loading, laser-induced stress may project longer distances than laser heating. For short pulsed laser irradiation, laser pulse duration and, laser exposure time should be longer than a minimum value to prevent cell damage in large areas of the tissue.

Brian J.F. Wong and his team have studied the damage of chondrocytes and cartilage matrix during laser reshaping of cartilage using Nd:YAG laser ($1.32\ \mu\text{m}$). They observed a direct relationship between laser dosimetry and tissue damage [48]. Level of laser-induced damage to the cartilage matrix and chondrocytes depends in particular, on laser wavelength. The use of an Erbium doped glass

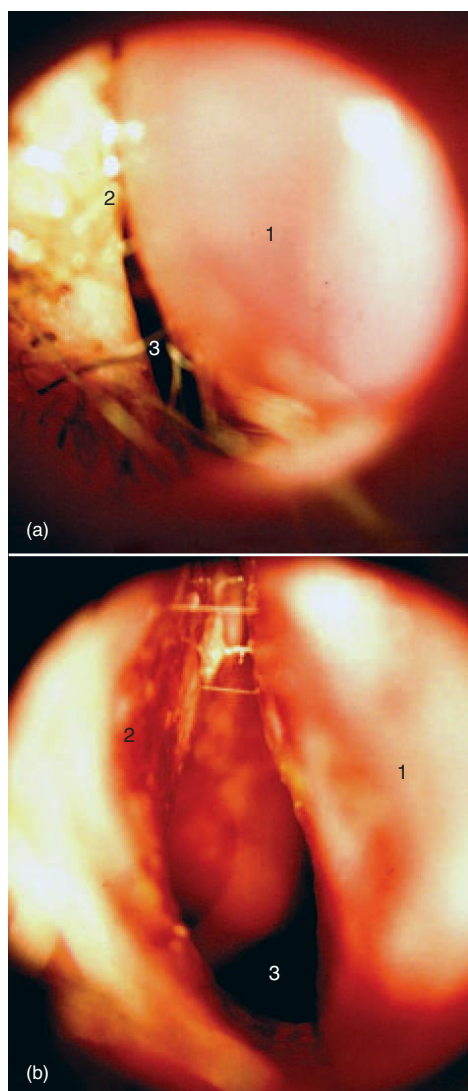


Figure 8 (online color at www.lphys.org) Rhinoscopic photograph, before (a) and 8 months after (b) laser septochondrocorrection procedure. 1 – septonasal cartilage; 2 – lateral wall of nasal cavity, 3 – general nasal cavity

fiber laser (1.56 μm radiation) allowed establishing the conditions of laser treatment resulting in stress relaxation without substantial damage to collagen, proteoglycans and to some extent chondrocytes.

For medical application of laser reshaping of cartilage – one can say that some chondrocyte death will not be a problem for successful treatment – compare with the knife where whole volumes of cartilage not only die but are removed permanently. Some chondrocyte death is acceptable as long as the entire tissue on the whole remains viable. We think the treatment may be optimized by minimizing volume of non-viable cartilage while still achieving shape change.

7. Mechanisms of laser reshaping

Various mechanisms for stress relaxation in cartilage have been discussed in the literature [2,3,6,49]. Early experiments indicated that laser-induced stress relaxation of cartilage has the characteristics of a type-I phase transition [2,22,50]. Although details of the phase transition are not fully understood, experimental evidence suggests the first step of this process is bound-to free phase transition of cartilaginous water [3,22]. Transition in water state may be followed by:

- I. local tissue mineralization (neutralization) of negative charged groups on proteoglycans by Na or Ca cations) without any changes in collagen and proteoglycan structure [3,25];
- II. local depolymerization of proteoglycan aggregates under short-term laser heating exceeding 70°C followed by the formation of a new proteoglycan structure without pronounced denaturation (dramatic structural changes) of the cartilage matrix [3];
- III. short-lived breaking of bonds between collagen and proteoglycan subsystems allowing decreased stress in cartilage by some alterations in the space structure of proteoglycans [3,6];
- IV. Formation of micropores in the cartilage matrix [49]. Micropore formation is a recently proposed mechanism for laser-induced stress relaxation in cartilaginous tissue and is discussed in the framework of spatial macroscopic heterogeneity of the structural and mechanical properties of the cartilage matrix, specifically, the existence of relatively strong regions (domains) separated by softer layers. Laser photothermolysis of these layers gives rise to domain mobility and induces micropore formation that may result in stress relaxation [6].

This mechanism, when considering heterogeneity of chemical bond breaking and structural changes in biopolymers requires adequate energy consumption and can provide long-term stability of cartilage shape. A simple mathematical model of this process [49] describes the qualitative features of laser-induced stress relaxation in cartilaginous tissue. The model is being refined to a more rigorous quantitative theory. Experimental data on micropore formation obtained using atomic force microscopy [25,45], optical coherence tomography and Raman spectroscopy [6] are consistent with the fundamental predictions of this model.

8. Animal studies of laser reshaping of cartilage

Research in physical and chemical properties of *ex vivo* cartilage specimens provided the basis for development of new laser methods in otolaryngology and cosmetology. Results obtained *ex vivo*, however, cannot be automatically

transferred to living tissues. In living tissues the long term equilibrium state of the tissue is a result of two processes: first that obtained after laser treatment, and second, after some time – due to tissue regeneration processes. Only *in vivo* experiments can provide adequate information on the response of living tissues to laser irradiation. First studies of laser reshaping of cartilage *in vivo* were performed on rabbit ears using CO₂ [46] and Holmium laser [51]. Three typical responses to laser irradiation were observed: coagulation necrosis, stable-state alteration of cartilage shape and temporary reshaping of cartilage.

One of the prospective applications of the technique of laser cartilage reshaping is the formation of cartilage implants. Shape stability of cartilage implants obtained from rabbit ear cartilage with CO₂ laser irradiation and then auto implanted under the skin of the same rabbits, was studied by Velegrakis et al. [52]. The experiments indicate that cartilage retained its new shape in 12 months after implantation. Optical and electron microscopy have shown viability of most chondrocytes in the irradiated zone. Chondrocytes had plural cytoplasmic processes and organoids are distinctive for elastic type cartilage. Cartilage matrix possessed non-damaged collagen fibers. The disadvantage of using CO₂ lasers ($\lambda = 10.6 \mu\text{m}$) for cartilage reshaping is the superficial character of light absorption results in overheating and destruction of superficial layers. Use of other laser wavelengths with deeper penetration allows more homogeneous heating without damage to superficial layers.

Reshaping of the collapsed tracheal cartilage of dogs was performed using 1.44 μm laser radiation of a Nd:YAG laser [53,54]. Reshaping of ear cartilage for the pigs was performed with a Holmium laser [15,47]. Reshaping of rabbit ear cartilage using Er:Glass 1.54 μm laser radiation was studied by Mordon [55].

For the ears, thin cartilage is covered by skin, and various techniques have been applied to avoid or minimize skin damage: skin incision with an optical fiber [6] and skin cooling [55]. Experimental results [6,55] indicate the conditions for *in vivo* laser reshaping are different from those found *in vitro*. Furthermore, histological examination of ear cartilage undergoing laser reshaping indicates a new cartilage of hyaline type replaces the old partially damaged irradiated cartilage of elastic type. We found that, in cases where stable changes in auricular shape were attained, elastic fibers were partially destroyed and did not reform in the regenerative zone [6]. Results obtained thus far indicate cartilage regeneration occurs more intensely near the area where laser radiation has caused some structural alteration or damage [47]. From these studies we hypothesize that regeneration of cartilage is probably stimulated by laser-induced stress redistribution and also by breaking some chemical bonds in the tissue.

9. Laser septochondrocorrection. Clinical work

Since 1999 the laser septochondrocorrection surgical procedure has been performed at the Ear- Nose-Throat Clinics of the I.M. Sechenov Moscow Medical Academy for 230 patients aged 9 to 75 years suffering from a deformed nasal septum. Treated patients were 132 males and 98 females. 12 patients had previously undergone the traditional surgical operation (perichondrium resection or septoplasty) to correct septonasal deformities. Before treatment, all patients complained of heavy breathing with symptoms including nasal discharge, chronic rhinitis, headache, nasal-ity, the chronic sinusitis.

The pulse-periodic Ho:YAG laser (wavelength of 2.09 μm ; pulse duration of 500 μs ; pulse energy of 0.2 – 0.4 J; pulse repetition rate of 5 Hz) was used for treatment of the first 110 patients, and an Erbium doped glass fiber laser (wavelength 1.56 μm ; power 3 W) was used for the following 120 patients.

An optothermomechanical contactor with transparent indenter and thermocouple sensor is placed at distal end of the optical fiber. When the contactor is inserted into the patient's nostril, the indenter compresses the nasal septum to extrude some water from the mucosa decreasing light absorption and preventing overheating and destruction. Septal regions of maximum mechanical stress are treated by laser irradiation. The temperature of cartilage is recorded by the feedback control system. Laser radiation is automatically switched off at the attainment of a preset temperature. Preset temperature is selected (depending on the type of the contactor and laser fluence) at 40 – 42°C at the periphery of the laser spot and about 55 – 60°C in the centrum of laser spot on the irradiated surface of septal cartilage. The irradiation of each spot takes a few second and does not result in damage of the mucosa and perichondrium. Laser septochondrocorrection is a painless and bloodless surgical procedure that requires 10-12 minutes to complete.

A survey of all patients was performed 2 – 3 times following treatment: following day, 2 – 3 weeks, and finally 6 – 8 months after administering the laser procedure. Rhinomanometry and nasal endoscopy were used to confirm the results of laser septochondrocorrection.

Enlargement of the nasal airway and improvement in nasal airflow have been achieved for all laser treated patients. Nasal septal shape remained stable throughout the observation period (up to 7 years). The degree of septal correction differed for Ho:YAG laser and Er doped glass fiber laser. Laser treated septonasal cartilage returned to (partly or fully) a deviated shape in 23% of cases for Ho:YAG laser and 10% of cases for Er-doped glass fiber laser. These patients that did not receive stable positive results underwent laser septochondrocorrection a second time with good results.

Laser septochondrocorrection procedure was administered to 23 children (17 boys and 6 girls) aged of 9 to 15 years with seriously labored breathing due to nasal septal

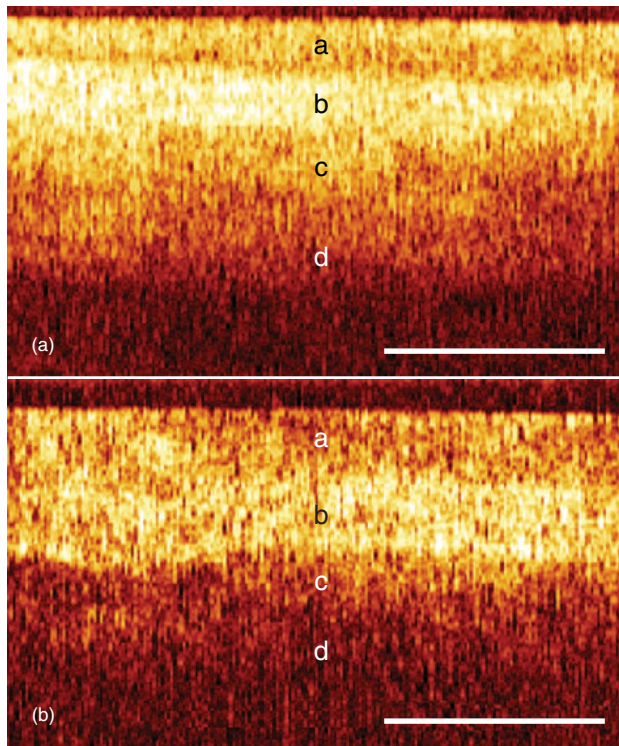


Figure 9 (online color at www.lphys.org) Optical coherence tomographic image of the human nasal septum (a) before and (b) 6 days after laser reshaping. a – mucosa; b – perichondrium; c – cartilage; d – deep layers of cartilage

deformations. These patients included complicated cases of a comb-shaped and S-shaped septum. Mechanical correction of deviated septum and a subsequent laser procedure were well tolerated for all patients. All young patients have shown a stable septal cartilage shape change and significant improvement in breathing. Follow-up observations over three years have not indicated any complications or secondary effects.

Nondestructive character of laser septochondrocorrection is demonstrated by optical coherence tomography (OCT) examinations of 24 patients a few days and six months after laser treatment. Representative OCT images (original size, 1.4×0.8 mm; resolution, $15 \mu\text{m}$) of the septal tissue before and 4 days after laser correction (in the same patient) are shown in Fig. 9. Before- and post-treatment images appear similar. The mucosa, perichondrium, and cartilage layers are identified, with contrast between each layer. Similar OCT images were also obtained when examining septonasal tissues at later dates after laser correction.

In contrast, OCT examination of nasal septum 6 days and 15 months after conventional septal surgery revealed considerable tissue alterations [6].

Medical equipment “Laser Septocorrector” LSC-701 (ARCUO MEDICAL, INC, USA) is developed and avail-

able commercially. The device utilizes an erbium-doped glass fiber laser with wavelength of $1.56 \mu\text{m}$. Laser radiation is delivered via a quartz fiber to the optothermo-mechanical contactor with a built-in transparent indenter and two thermocouple sensors. A specialized instrument is used to mechanically straighten the deviated septum during laser treatment. The original feedback control system based on the monitoring of temperature signals during the course of laser irradiation of septonasal cartilage is applied to insure safe and efficient application of the laser procedure. The clinical trials of LSC-701 have shown:

- (a) straightening of nasal septum was obtained for all patients immediately after laser treatment;
- (b) most patients (90%) showed a steady-state breathing improvement at one year follow-up;
- (c) good procedure tolerance for all patients;
- (d) no complications and secondary effects were observed in any patients. No injuries in the mucosa, perichondrium, cartilage and surrounding tissues were observed.

10. Application in cosmetic plastic surgery

Laser reshaping of cartilage can be used for cosmetic shape correction of the ears and nose wings. *Ex vivo* and *in vivo* experimental studies of laser reshaping of rabbit and porcine ears were reported [1,3,6,22,47,55–57].

These studies have established the requisite laser dosimetry to obtain a stable shape re-configuration of porcine ears [6]. Clinical potential to reshape protruding ears by neither cutting skin nor scoring cartilage without anesthesia was reported [57]. Eight patients have been treated successfully using a $1.54 \mu\text{m}$ Er:Glass laser to correct shape of ear cartilage. The laser treatment was well tolerated by patients, did not cause pain nor lead to any adverse secondary effects. Degree of correction and stability appeared similar to that obtained by conventional transection or cartilage weakening techniques. Furthermore, the laser surgical treatment can produce a smoother and more natural curvature than conventional techniques.

Experimental study of laser reshaping of nasal wings using $1.56 \mu\text{m}$ fiber laser was performed first in porcine cartilage and then for human cartilage taken from volunteer’s nose during conventional cosmetic plastic surgery [6]. The laser dosimetry established allowing a desired shape change of the nasal wings.

11. Laser regeneration. *In vivo* animal studies

Regeneration is an essential response of tissue to mechanical, thermal or chemical damage [58]. Studies of the effect of non-destructive laser irradiation on cartilage regeneration were reported [5,6,59–61]. Objective was to study effect of laser irradiation on repair of intervertebral discs and to establish laser dosimetry for growth of new cartilage or

bony tissue. Rabbit intervertebral discs have been irradiated *in vivo* using a 1.56 μm fiber laser with various pulse durations (from 10 ms to 2 s) and repetition rates (from 0.3 to 2 Hz). In the operating suite, laser radiation was transmitted through a 400 μm quartz fiber into the NP by puncturing the disc using a 1 mm diameter needle. Conventional histological technique and AFM have been used to examine morphological changes in intervertebral discs. These studies have demonstrated that laser irradiation of intervertebral discs can induce metaplasia of fibrous cartilage into hyaline type cartilage. Laser irradiation to generate hyaline cartilage in human intervertebral discs has been demonstrated *in vivo* for the first time [61].

Experimental studies have been completed prior to clinical tests both *in vivo* (on the 36 rabbit spine discs) and *ex vivo* (on two cadaver's spines) [5]. Temperature dynamics during the course of laser irradiation of intervertebral discs has been monitored. Results of these studies demonstrate the locality and safety of laser treatment of intervertebral discs. The histological evaluation of rabbit intervertebral discs two and three months after laser exposure demonstrated the growth of new cartilaginous tissue. Images recorded by AFM show that nondestructive laser irradiation provokes formation of micropores in cartilaginous matrix. Micropores promote water permeability and increase the flow of nutrients to chondrocytes. Endoscopic imaging shows that laser irradiation results in mechanical oscillation of the NP in the rabbit and cadaver intervertebral discs [6]. Mechanical oscillations of specific frequencies and amplitudes are known to lead to activation of chondrocytes metabolism [62,63]. We hypothesize that laser induced mechanical oscillation and micropore formation in the spine disc cartilages may promote regeneration processes.

Morphological study of rabbit intervertebral discs irradiated using different laser dosimetry was performed using optical and electron microscopy at 3, 30, and 90 days after irradiation. Structural alterations observed in rabbit intervertebral discs 3 days after irradiation showed areas of (a) mechanical necrosis from needle puncture, and (b) laser necrosis due to thermal and mechanical effects of laser irradiation. Volume of necrotic tissue depends on laser dosimetry, and laser dosimetry exists that produces very small volumes of cell damage. Indicators of the beginning of regeneration process can be observed on the periphery of the small necrotic zones. Large chondrocytes and multicellular clones appear. Accumulation of glycosaminoglycans is observed surrounding active chondroblasts and clones.

At one and three months after laser irradiation, the most pronounced signs of regeneration are observed in the inner layers of annulus fibrosus (AF) and in the NP. The microphotographs of a rabbit intervertebral disc in three months after laser irradiation using an 1.56 μm fiber laser is shown on the Fig. 10. A number of active chondrocytes are surrounded with typical lacunae. Young chondroblasts, large isogenic groups can be seen on the Fig. 10a.

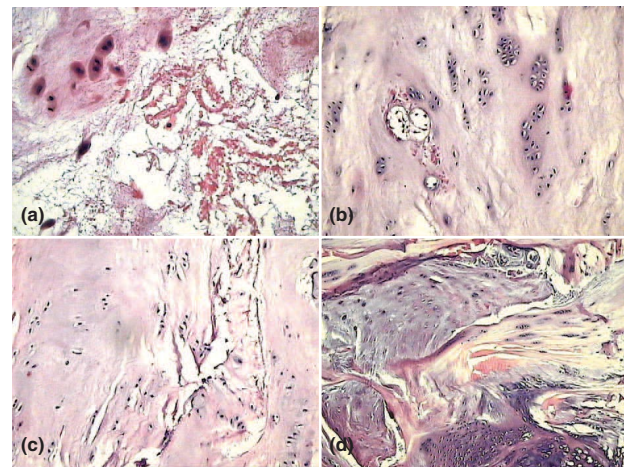


Figure 10 (online color at www.lphys.org) The microphotograph of a rabbit intervertebral disc in three months after laser irradiation using an 1.56 μm fiber laser (Stained with Hematoxylin and Eosin). (a) – Young chondroblasts, large isogenic groups, and a small necrotic zone in the inner layer of the AF ($\times 400$); (b) – Neogenic tissue replaces the necrotic zones. Multi cellular clones are seen ($\times 200$); (c) – Transformation of fibrous-hyaline to hyaline type cartilage ($\times 200$); (d) – Mosaic structure of neogenic tissue growing in the NP including zones of fibrous, fibrous-hyaline and hyaline cartilage ($\times 100$)

Two-three cell and multi cellular clones are seen on the Fig. 10b. Neogenic tissue replaces the necrotic zones, and grows beyond the boundary. Neogenic tissue has features both of fibrous and hyaline cartilage: the shape and ultrastructure of chondrocytes are similar to cells in hyaline cartilage, but the structure of intercellular matrix possesses the random (disorderly) distribution of thin collagen fibrils like hyaline cartilage, as well as more thick and more aligned collagen fibrils observed in fibrous cartilage. This cartilaginous tissue is termed fibrous-hyaline cartilage (Fig. 10c). Apparently, its origin is poorly understood.

Chondroblasts become activated as a result of laser irradiation. In addition, regions of typical hyaline type cartilage are observed with homogeneous matrix structure and lacunae surrounding chondrocytes. (Fig. 10c). In the NP, dimensions of the necrotic zone decrease, and necrotic tissue is replaced with neogenic cartilage of hyaline or fibrous-hyaline types. The structure of non-damaged areas of NP also changes: multicellular clusters decrease in size, notochordal cells are progressively replaced by chondrocyte-like cells, and the bulbous-like structure of dense matrix surrounds residual notochordal cells. TEM shows zebra like corpuscles and spiroid proteoglycan structures. Gradually the clusters in the NP disappear, the matrix condenses and becomes fibrillar or granular in structure. NP took on a mosaic appearance (Fig. 10d). Bulk ratio of the different structural zones and the degree of regeneration depend on the laser dosimetry and relative

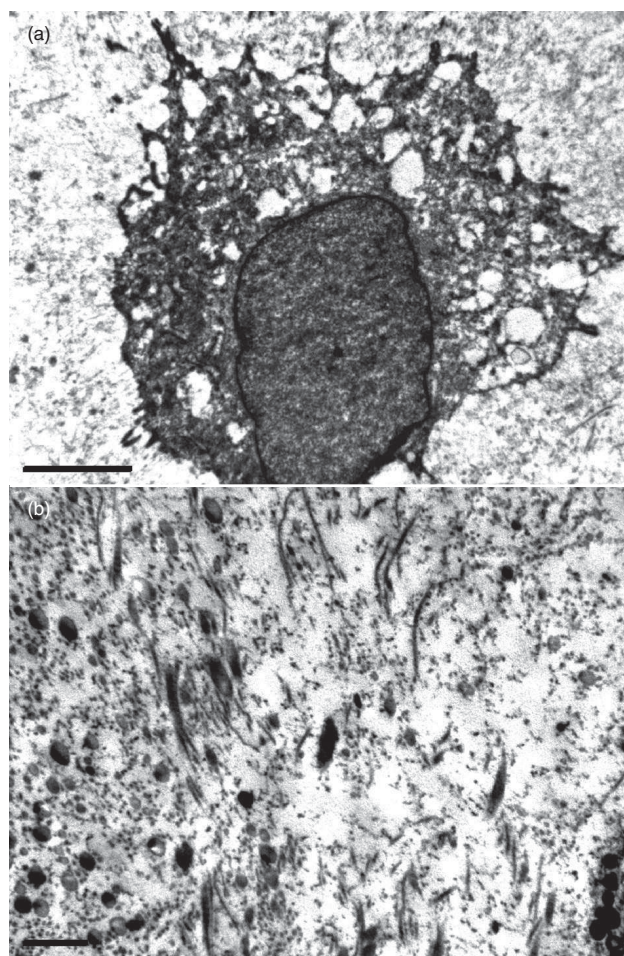


Figure 11 TEM micrograph of the rabbit intervertebral disc three months after $1.56 \mu\text{m}$ laser irradiation. ($\times 30000$). (a) – Active chondrocyte in the fibrous-hyaline cartilage grown in the irradiated zone of the AF; (b) – Matrix of new growing hyaline-type cartilage. Thin disorderly oriented collagen fibrils and proteoglycan granules are observed

position of the laser treated zones. Fig. 11 presents a TEM micrograph of a rabbit intervertebral disc three months after $1.56 \mu\text{m}$ laser irradiation.

Active chondrocytes in the fibrous-hyaline cartilage are observed in the irradiated zone of the discs. Matrix of new growing hyaline-like cartilage is shown in Fig. 11b.

Up to the third month after laser treatment, the transformation of fibrous cartilage to bony like tissue appeared on the external AF surface. Mineralization and ossification may be due to high mechanical stress and damage to AF tissue due to the needle incision followed by laser irradiation. Mineralization and ossification may have a positive clinical effect by decreasing mechanical instability of intervertebral discs.

The results of laser-induced regeneration for rabbit intervertebral discs indicate optimal laser dosimetry exists

that allows regeneration of hyaline-like cartilage in the inner regions of the AF and NP and bony tissues in the external regions of the AF damaged zones. Non-ablative laser irradiation modifies the tissue structure and may promote growth of new tissue with a pronounced heterogeneous structure. Although further studies are required, the neogenic tissue may provide a tough, elastic and functional component in intervertebral discs.

12. Laser reconstruction of discs. Clinical work

Laser reconstruction of intervertebral discs (LRD) is a novel and minimally invasive surgical approach for the treatment of intervertebral disc diseases. The technique uses local, non-destructive laser irradiation for the purpose controlled activation of regenerative processes in laser treated zones while minimizing undesirable effects on adjacent tissue regions. The procedure may be performed in an outpatient setting requiring only thirty minutes to complete without the need for general anesthesia [6,61]. The scheme of the LRD procedure is shown schematically in Fig. 12. LRD is performed using needle puncture of the damaged disc followed by irradiation of the NP with Er-doped glass fiber laser using precise and controlled laser dosimetry. The position of the needle with an optical fiber is controlled with X-ray imaging (Fig. 13).

Laser treatment leads to activation of tissue regenerative processes, and neogenic cartilaginous tissue grows in laser-treated zones.

Since 2001, LRD has been performed for 205 patients suffering from chronic symptoms of low back or neck pain who failed to improve with non-surgical therapy. Duration of pre-LRD symptoms was more than 1 year, average patient age was 45 (ranging between 22 and 64) and average follow-up period was 12 months.

Pre-op and post-op assessment included X-ray and MRI imaging. Provocative discography was applied for the determination of number of levels to be treated by LRD. The post-op discography examination allowed imaging neogenic dense tissue (arrow) filling the disc defect (Fig. 14). The AF became more dense and homogeneous, and the defect decreased in volume. The result demonstrates the reparative dynamic in intervertebral discs after non-ablative laser irradiation. All patients undergoing LRD demonstrated significant pain relief, improvement of walking, personal care, and general comfort. In all patients with discogenic neck pain, other symptoms such as attacks of dizziness also diminished. Substantial relief of back pain was obtained in 90% of patients treated who returned to their daily activities with many participating in recreational sports.

Mechanisms of laser-induced regeneration of hyaline type cartilage in the intervertebral discs are under investigation. Possible mechanisms include [6,61]:

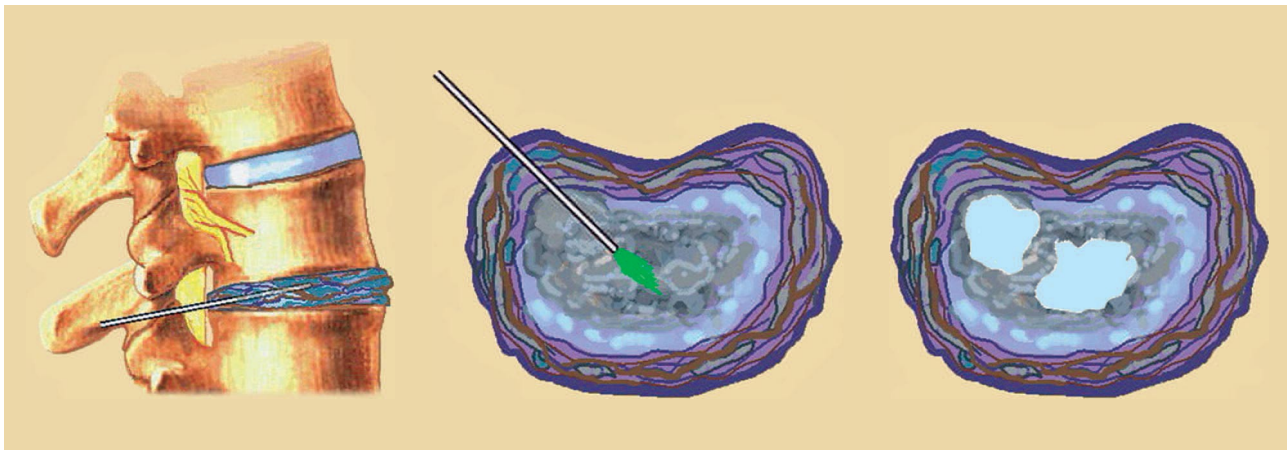


Figure 12 (online color at www.lphys.org) Diagram for laser reconstruction of intervertebral discs (LRD)



Figure 13 X-ray control for positioning needle with an optical fiber during LRD for a patient

- (1) Space-time modulated laser irradiation induces inhomogeneous and pulse repetitive thermal expansion and stress in cartilage. Mechanical effect due to controllable thermal expansion of the tissue and micro and nano-bubble formation in the course of moderate (up to 45 – 50°C) heating of the NP activate cells and promote cartilage regeneration. Previous studies indicate

dynamic mechanical oscillations may activate tissue regeneration [62,63];

- (2) Non-destructive laser irradiation leads to the formation of nano- and micro-pores in the cartilage matrix. Pore formation promotes water permeability and increases flow of nutrients to chondrocytes;
- (3) Increasing of **Ca** concentration near the cells due to transfer of water from the compressed to expanded areas of the cartilage matrix. Water carries solutes with positive ions of **Na** and **Ca**. Since **Na** ions are lighter and leave the compressed zone more rapidly than **Ca** ions, the **Ca** concentration increases in the compressed areas and may activate metabolic processes and promote tissue regeneration.

An open question is origin of chondrocytes that produce hyaline-type cartilage. Three sources for observed chondrocytes are recognized: (1) chondrocytes may diffuse from hyaline plates (far from the area of regeneration, but it is known that laser radiation may accelerate diffusion processes [2]), (2) specialization of stem cells, and (3) activation of biosynthesis and proliferation of chondrocytes in the inner part of AF. Future research is aimed to clarify this important question.

13. Conclusions

We have developed a **new approach** using nondestructive moderate laser irradiation to modify the ultra structure, thermal and mechanical stress fields in tissue. Space-time modulated laser radiation allows controlled modification of the stress distribution in cartilaginous tissues. Thermal-mechanical effects may result in controlled alteration of cartilage shape and activation of regenerative processes.

Thermal and mechanical effects of laser radiation have been used for tissue modification. Advantages of lasers compared with other energy sources include the local and

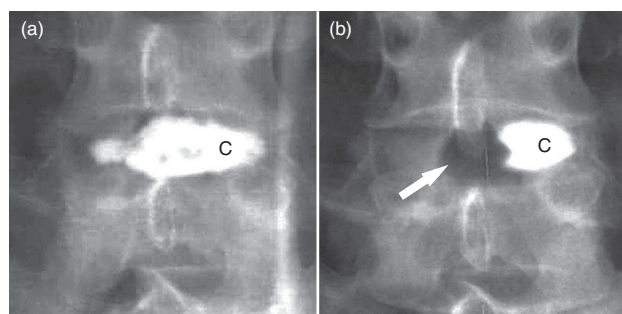


Figure 14 Discography of a patient (a) before and (b) five months after LRD. Filling of the AF with the neogenic tissue is evident (arrow)

precise control of the laser – material interaction. Lasers are very convenient for energy delivery to a specific location and also for feedback control of the optical, thermal and mechanical effects in tissue for effective and safe treatment while minimizing non-desirable effects.

Reshaping of nasal septal cartilage under non-destructive laser irradiation is a demonstrably novel, effective and safe application of lasers in otolaryngology. In comparison with the conventional surgical procedure using mechanical tools, laser septochondrocorrection is a bloodless, painless, out-patient, noninvasive, repeatable, fast and inexpensive procedure that may be applied to young and old patients alike. Laser reconstruction of degenerated intervertebral discs is a novel minimally invasive approach using local, non-destructive laser irradiation for controlled activation of regenerative processes in the laser treated zone without any damage to adjacent tissue regions. Advantages of this approach compared to other techniques based on heating of intervertebral discs are the principal role of mechanical effects of laser radiation with minimal heating of cartilage, minimal effect on the annulus fibrosus and the nerves in the superficial layers of the discs, the controllable character of tissue modification and regeneration resulting in the growth of hyaline type cartilage in the irradiated zone. Clinical trials of novel laser technologies and equipment have been performed in Russia for over 400 patients. Clinical trials demonstrating efficacy of this technology are planned for Europe and the United States.

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