20
Laser Reshaping of Cartilage

EMIL SOBOL[1]*, ALEXANDER SVIRIDOV[1], ALEXANDER OMEL'CHENKO[1], VICTOR BAGRATASHVILI[1], MOISHE KITAI[1], STEPHEN E. HARDING[2], NICHOLAS JONES[3], KORNELIA JUMEL[2], MICHAEL MERTIG[4], WOLFGANG POMPE[4], YURI OVCHINNIKOV[5], ANATOLY SHEKHTER[5] AND VALERII SVISTUSHKIN[5]

[1]*Institute of Laser and Information Technologies, Russian Academy of Sciences, 142092 Troitsk, Russia,* [2]*University of Nottingham, NCMH unit, School of Biological Sciences, Sutton Bonington, Leics. LE12 5RD, U.K.,* [3]*University of Nottingham. Division of Otorhinolaryngology, Queens Medical Centre, Nottingham NG7 2UH, U.K.,* [4]*Technical University of Dresden, Institut fur Werkstoffwissenschaft, D-01062 Dresden, Germany and* [5]*Sechenov Medical Academy of Moscow, Moscow 119881, Russia*

Introduction

Cartilage is a dense connective tissue, composed of 60–80% water containing a small proportion of cells (chondrocytes) in an extracellular matrix (ECM). The ECM is a hydrated gel containing proteoglycans that rely on the diffusive transport of water through the cartilage for both function and to maintain its nutrition. Because cartilage is avascular, then autologous grafts will resist resorption by the host organism (Caplan, 1984). Although various treatment strategies have been proposed for the repair or regeneration of deformed and diseased cartilage (Donald, 1992), the development of a reliable therapeutic has been elusive. Inasmuch as destruction of cartilage results in crippling disability, an urgent need therefore exists for the development of novel therapies for the repair and regeneration of deformed and diseased cartilage. Because the efficacy of cartilage in transplantation purposes depends specifically on the availability of tissue with the appropriate size and shape, the technique of laser-assisted reshaping may find important applications in, for example, otorhinolaryngology, orthopaedics, and plastic surgery.

Although ablation of cartilaginous tissue has been documented by various

*To whom correspondence may be addressed (sobol@laser.ru)

Abbreviations: ECM, extracellular matrix; PG, proteoglycan; CS, chondroitin sulphate; PBS, phosphate buffered saline; SEC, size exclusion chromatography; MALLS, multi-angle laser light scattering; AFM, atomic force microscope; EDX, electron diffraction/X-ray analysis; FTIR, Fourier Transform Infra Red spectroscopy.

Biotechnology and Genetic Engineering Reviews – Vol. 17, August 2000
0264–8725/00/17/553–578 $20.00 + $0.00 © Intercept Ltd, P.O. Box 716, Andover, Hampshire SP10 1YG, U.K.

investigators (Vangsness and Chaden, 1993), our team pioneered the first successful demonstration of laser-assisted cartilage reshaping (Helidonis *et al.*, 1993; Sobol *et al.*, 1994). Additionally, we identified the primary biophysical process. The phenomenon of stress relaxation and reshaping of cartilage under laser radiation is described in papers by Helidonis *et al.* (1994), Sobol (1995) and Sobol *et al.* (1994, 1996a). It was established that laser-assisted cartilage reshaping is dependent on a temperature sensitive, bound-to-free water phase transition (Sobol, 1995; Sobol *et al.*, 1997; Bagratashvili *et al.*, 1997). Under ordinary conditions, mechanical resistance to cartilage reshaping originates in the large molecular forces between water and proteoglycan (PG) molecules. Under moderate laser heating, the internal stress normally present in the cartilage is momentarily reduced when water bound to the PGs is liberated. If the bound-to-free water phase transition is effected without damage to the surrounding protein or carbohydrate molecules, then stable modified cartilage configurations may be achieved. It was shown that the process of laser-assisted cartilage reshaping is accompanied by changes in the mechanical, thermal, and the optical properties of the tissue; changes which can be measured (Omel'chenko *et al.*, 1999a; Sobol, 1995; Sobol *et al.*, 1996a; Sviridov *et al.*, 1996; Wong *et al.*, 1997, 1998a).

The first laboratory demonstration of laser-assisted reshaping of cartilage was obtained using radiation (10.6 μm) emitted from a CO_2 laser (Helidonis *et al.*, 1993; Sobol *et al.*, 1994; Sobol, 1995; Velegrakis *et al.*, 1994). Later, studies utilizing a Ho:YAG laser indicated that 2.1 μm (wavelength) radiation allows reshaping of 1 mm thick cartilage specimens without overheating or destruction of structures near the tissue surface (Sobol *et al.*, 1996b; Sviridov *et al.*, 1998). Alternative laser wavelengths of 1.44 μm (Wang *et al.*, 1995, 1996) and 1.32 μm (Wong *et al.*, 1997, 1998b) have been used successfully for reshaping of deformed cartilage. *In vivo* animal experiments have shown that it is possible to get a new stable configuration of porcine ear cartilage while there are three different time-scales of the stability of a new shape obtained: days, weeks and 'permanent' (ie for a period of at least three months). The results of operations on the nasal septum of 40 patients performed at the Medical Academy of Moscow in 1998 have been very promising. A non-destructive laser treatment of a bovine knee capsule has also recently been reported and attributed to collagen denaturation (Naseef *et al.*, 1997). More recently, the clinical results of non-ablative laser shrinkage and tightening of intervertebral and hermated cervical spinal disks have been documented (Chiu *et al.*, 1998). Thus, non-destructive laser reshaping and treatment of a deformed cartilage has the potential to become a new powerful application of lasers in medicine.

In this review, we examine the progress that has been – and is being – made, and our state-of-the-art understanding of the mechanism of laser-induced stress relaxation in cartilage, and present recent results in laser reshaping of cartilage *in vivo*.

Laser modification *in vitro* of cartilage shape

When one tries to give cartilage a new shape, the arising stress returns the tissue to its initial configuration. Local irradiation of area of maximal stress leads to stress relaxation, and results in a stable configuration of cartilaginous tissue (*Figure 20.1*). An example of a porcine ear reshaped *in vivo* under laser radiation is shown on *Figure 20.2*.

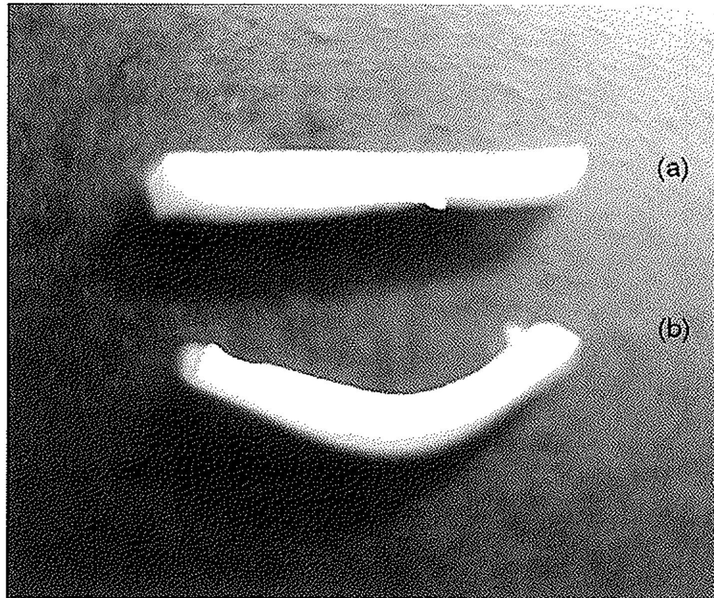


Figure 20.1. Modification of cartilage shape: (a) before, (b) after laser treatment (Sobol, 1995).

Figure 20.2. Porcine ear in three months after the laser reshaping (Sviridov *et al.*, 1999).

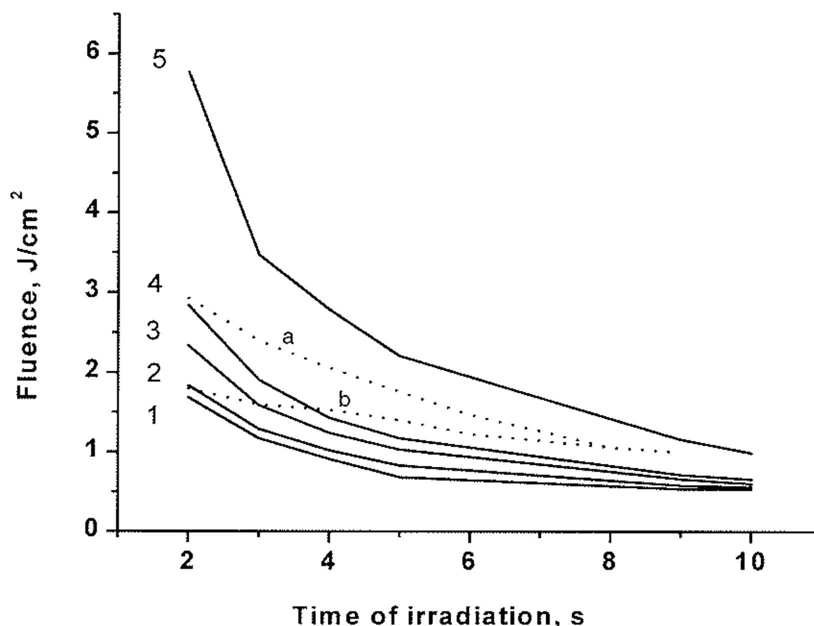


Figure 20.3. Optimal condition for cartilage reshaping with a Holmium laser. (Sobol *et al.*, 1999a). Laser fluence (flux density) vs. exposure time for various values of diffusion length: l = 1.2 mm, $\alpha = 40\ cm^{-1}$, f = 5 Hz. Solid curves are calculations for (1) L = 2 μ, (2) 6 μ, (3) 12 μ, (4) 15 μ, (5) 25 μ. Dashed curves correspond to experimental results obtained with a Holmium laser: (a) threshold for denaturation (upper dotted line), (b) threshold for stress relaxation (lower dotted line).

No dramatic structural alterations in the tissue were observed using the conventional histological technique for some regimes of laser reshaping of cartilage. The structural integrity of the tissue appears to survive when the laser energy and exposure times are not too large.

There are conditions for cartilage reshaping *in vitro* which produce no pronounced changes in the matrix structure as viewed by light microscopy (Sviridov *et al.*, 1998). In comparison to the effects (on the basis of histological analysis) on cartilage reshaped by laser radiation with wavelengths of 10.6 μm (Helidonos *et al.*, 1994, 1995; Ovchinnikov, 1995) or 1.44 μm (Wang *et al.*, 1995, 1996), observations by Sviridov *et al.* (1998) on the effects of a Holmium laser define a 'window of energy density' which represents laser reshaping without damaging the cartilage matrix (*Figure 20.3*). It is seen that, for pieces of cartilage of 1 mm in thickness, a laser treatment time of 4 seconds can be sufficient to allow stress relaxation to occur, but an energy density of less than 2.2 J/cm^2 is inadequate to cause a significant alteration in cartilage structure. The '*window*' between the conditions needed for laser reshaping and those that produce structural changes in the cartilage matrix decreases with treatment time. A treatment time, for example, of 9 seconds produces laser shaping, but it is accompanied by pronounced changes in the structure. This phenomenon can easily be understood in terms of the process of PG denaturation or degradation taking more time than that required for the stress relaxation process which occurs as a result of the reorganization and short-range movement of water molecules. The use of a different cartilage thickness, type of laser or other conditions may alter the '*window*'

at which stress relaxation occurs, and the same time avoids producing tissue damage. In particular, our recent experiments with a 1.56 μm laser radiation (Sviridov *et al.*, unpublished) have demonstrated that an exposure time of 0.5 s is sufficient to produce a new stable configuration of cartilage of 0.5 mm in thickness. The optical properties of cartilage *in vitro* can differ from that of living tissue, and also depend on the age and type of cartilage. The optimum conditions for laser treatment have been investigated by *in vivo* studies, as we will consider below.

THE EFFECT OF LASER IRRADIATION ON CHONDROCYTES

Chondrocytes are more sensitive to damage by laser treatment than the cartilage matrix. In the experiments of Sviridov *et al.* (1998), laser shaping with a holmium laser was accompanied by some alterations in chondrocyte morphology. Two types of cell alteration were distinguished: cytoplasmic focal vacuolation (FV), which may be associated with reversible cell injury, and nuclear condensation (NC), which is generally regarded as being indicative of cell death. The histological changes in chondrocytes have been quantified using a semi-quantitative rating of severity according to the proportion of cells affected.

However, Sviridov *et al.* (1998) also showed that there are conditions (such as a laser 'fluence' (flux density) of 1.7 J/cm^2 at an exposure time of 4 s) which allow laser shaping without nuclear condensation and only produce minor cell vacuolation. Laser heating with lower values for these parameters produces only minimal cell damage but, unfortunately, no laser shaping. If the flux in laser energy is increased or the treatment time prolonged, significant nuclear condensation occurs. Histopathological analysis has also revealed that the amount of chondrocyte damage varies throughout the thickness of the cartilage: the more superficial cells showed morphological evidence of damage, whilst many of the cells in the deeper main body of the cartilage remained undamaged. The effect of cytoplasmic vacuolation alone on the long-term integrity of the cartilage is not known, and an *in vivo* study is planned to examine this.

The possibility that laser energy might stimulate chondrocyte metabolism cannot be ruled out. This question has been studied by several workers in connection with arthritis, but the results have been rather contradictory. Pullin *et al.* (1996) have found chondrocyte formation close to a 'crater' which had been created by a holmium laser in the articular cartilage of horses *in vivo*, but regeneration of cartilage was not reported. Dillingham *et al.* (1993) found a substantial reduction in the inflammation of the synovial region when an articular lesion was treated with holmium laser radiation. Sato *et al.* (1999) studied the effect of laser radiation on chondrocytes when suspended in solution. These workers discovered that there is an optimal amount of laser energy corresponding to the maximal content of chondrocytes in the solution. Chao *et al.* (unpublished) have studied the effect of laser radiation on chondrocytes by using an isotope technique. Laser irradiated cartilage grafts were evaluated for viability by measuring the incorporation of sodium^{35} sulphate into proteoglycan (PG) macromolecules in whole tissue culture. PG synthesis rates were determined by measuring scintillation counts in lyophilized specimens and normalizing these values for the total protein content of each specimen. It was shown by Sato *et al.* (1999) that PG synthesis rates decreased with increasing laser exposure, and were always higher than baseline rates for nitrogen monoxide treated tissue. Sviridov *et al.* (1999) have

shown that laser radiation might activate regeneration processes in the tissue. The mechanism through which laser irradiation affects chondrocytes is not yet fully understood. It could be postulated that laser radiation could influence the pore structure, water permeability of the ECM, and the rate and efficacy of chondrocyte nutrition. It is known also that biological cells are sensitive to environmental conditions, in particular to the temperature and stress distribution. Since laser radiation induces a local heating and stress relaxation, it alters temperature and stress distribution and can affect chondrocyte function.

One of the reasons for cartilage degradation is cell death due to a decrease in the permeability of the ECM. There is some preliminary data (Sobol *et al.*, unpublished) which shows that irradiation of rabbit and porcine cartilage leads to increased cell proliferation, the formation of chondrocyte clusters within the tissue, and the growth of the new cartilage of the 'hyaline type' in the laser treated zone. This phenomenon may be caused by changes in the water diffusion kinetics in cartilage and the relaxation of the ECM. Cell surface receptors can detect small changes in their external environment, which could lead to changes in the metabolic and proliferative activity of the cells.

Thermal, mechanical, and optical effects

The study of the physical processes underlying the laser–induced stress relaxation has shown a number of thermal, optical and mechanical effects accompanying laser reshaping of cartilage:

(1) Calorimetric measurements reveal a peak at ~ 70°C which disappears when the sample is examined for the second time, and appears once again when the sample is examined after keeping immersed in a water bath for 20 minutes (Bagratashvili *et al.*, 1997; Wong *et al.*, 1999).
(2) A change of slope of the heating curve is evident at 70°C (*Figure 20.4*). When the rate of laser heating is not very high, the time dependence of temperature of cartilage being heated with constant laser power density is nearly constant at a temperature of ~ 70°C (Sobol *et al.*, 1994; Sobol, 1995). From the duration of this constant section in the heating curve, these workers estimated the value of energy requirement of such a transition to be between 1,200 and 1,800 J/cm^3. It was postulated (Sobol *et al.*, 1994; Sobol, 1995) that this energy consumption represents the transition of water from a bound to a free state.
(3) Laser-induced changes of the slope of a 'stress-strain' curve which manifests a decrease in cartilage elasticity (Omel'chenko *et al.*, 1999a).
(4) A maximum at 70°C for the temperature dependence of the internal friction in cartilage undergoing forced oscillation (Sobol *et al.*, 1996a, 1997).
(5) Laser radiation accelerates a stress drop in cartilage when the temperature reaches 70°C (Sviridov *et al.*, 1998; Wong *et al.*, 1997; *Figure 20.4*). In the early stages of the laser treatment process, the increase in stress may be due to tissue expansion under heating when the water being irradiated does not have enough time to diffuse to the periphery of the laser spot ('water inertial effect'). After a few seconds, the movement of water leads to a redistribution of stress and results in stress relaxation.

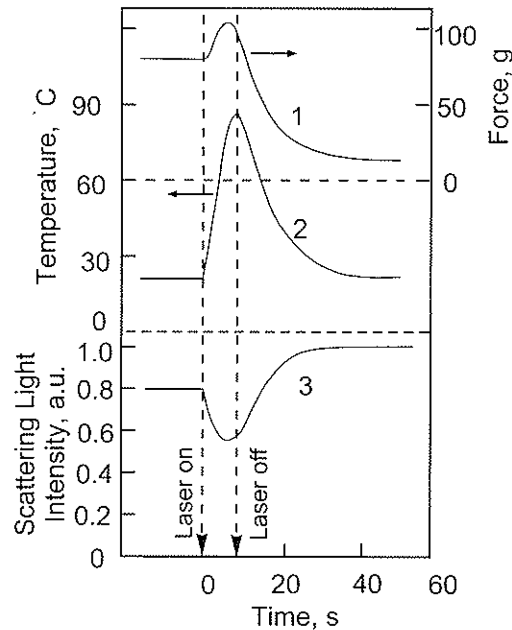


Figure 20.4. Temperature, stress and light scattering in cartilage vs. time of irradiation (Wong *et al.*, 1997, 1998b).

(6) Opto-acoustic response of cartilaginous tissue alters under laser radiation due to alterations in tissue mechanical properties (Omel'chenko *et al.*, 1999b). *Figure 20.5* shows the acoustic signal to a single laser pulse (a) at the beginning of the laser treatment, and (b) after the tissue temperature has exceeded 70°C and stress relaxation has occurred.

(7) Light scattering kinetics in cartilage being irradiated demonstrates an increase (a change of the sign of the slope) when stress begins to drop (Sviridov *et al.*, 1996; Wong *et al.*, 1998a,b). This may arise from the nucleus of a new phase (water droplets, micropores) that increases the number of light scattering centres.

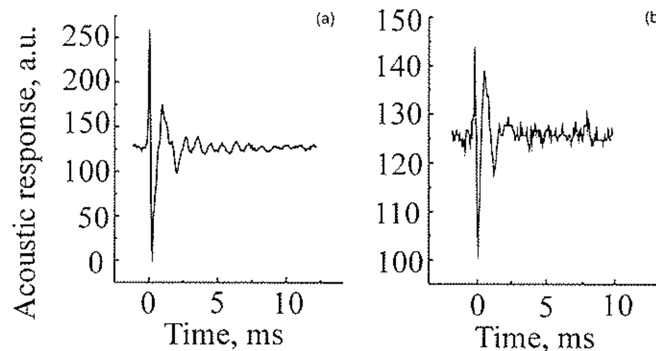


Figure 20.5. Acoustic responds of cartilage irradiated with a pulse Holmium laser (a) at the beginning of laser irradiation; (b) at the end of laser treatment (Omel'chenko *et al.*, 1999b).

It can therefore be deduced from these studies that there is a strong correlation between temperature, stress and light scattering detected during laser reshaping of cartilage. The process of laser-induced stress relaxation in cartilage appears like a phase transformation of the first kind, and there is evidence that this transformation is connected with a transition in cartilaginous water.

Examination of the fine structure of cartilage

The fine structure of cartilage and its alterations under laser radiation has been examined by atomic force microscopy (AFM) (see Mertig *et al.*, 1997b; Sobol *et al.*, 2000a). In these studies, the surface of cartilage samples have been imaged with a commercial multimode microscope, NanoScope IIIa with phase extender (Digital Instruments, Santa Barbara) operated in tapping mode at about 300 kHz. Scanning with a scan rate of about 1 Hz had been performed with silicon tip cantilevers (NanoProbe™, 125 mm) applying tapping amplitude between 30 nm and 50 nm. The main components of the ECM collagen fibres and proteoglycan 'clouds' are shown in *Figure 20.6* (a). Examination by AFM (Sobol *et al.*, 2000a) has shown that, depending on the laser treatment conditions, various peculiarities of irradiated structure can arise: for example, micropores (*Figure 20.6* (b)), which could be traces of PGs detached as a result of laser radiation and small sub-micron crystals (*Figure 20.6* (c)), which are not observed in normal, non-irradiated tissue.

A direct comparison of irradiated and non-irradiated tissue clearly indicates that short-time irradiation produces little change in the surface structure of cartilage (Sobol *et al.*, 2000a). The only observed change that is characteristic of all the samples that have been investigated in this way (Sobol *et al.*, 2000a) is the additional appearance of channels of 100 nm–400 nm in size which present themselves as holes and ditches in cut sections from the cartilage. The corresponding phase mode images exhibit clear thread-shaped structures with an average lateral width (calculated from the power spectral density of the images) of about 80 nm. It could be supposed that the observed features correspond to the fully hydrated core-proteins of the proteoglycan acid aggregates (Comper, 1991).

When the exposure time was increased up to 30 s, Sobol *et al.* (2000a) found that the cartilage structure appeared to have significantly changed in comparison to both untreated samples and also to samples exposed to 3 s laser irradiation. The characteristic proteoglycan morphology could no longer be observed with the sample appearing unstructured, indicating thermal destruction of the cartilage. For some samples irradiated *in vitro*, small crystals of 100 nm to 800 nm in size were also found by these workers (*Figure 20.6* (c)). Electron diffraction/X-ray analysis (EDX) of the chemical composition of these crystals demonstrated that they consist mainly of sodium carbonate. This is not chemically stable and so the crystals formed should eventually re-dissolve. Small crystals of a few hundred nm in size were also observed for cartilage samples four months after *in vivo* irradiation for 3.5 s: the extra stability here indicates crystals of a calcium rather than a sodium salt.

Usually, the process of mineral formation in cartilaginous tissue is strongly inhibited. However, it has been shown that local destruction of the proteoglycan structure can cause subsequent mineralization of cartilage (Poole, 1987). This process can be initiated artificially by chemical means, or may occur naturally as a result of

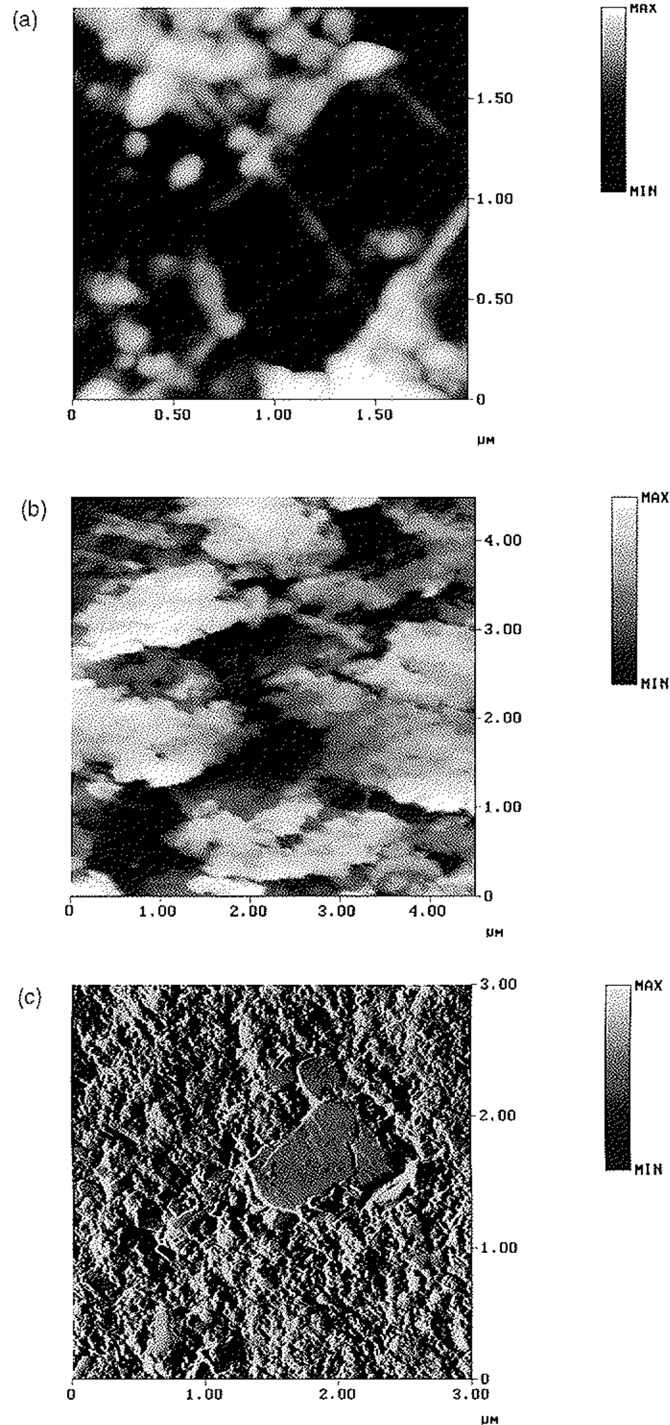


Figure 20.6. AFM pictures: (a) initial, collagen fibres and proteoglycan clouds are seen; (b) irradiated for 3 s, pores are seen; (c) irradiated for 30 s, crystals are seen (Sobol *et al.*, 2000a).

lysing the hyaluronic acid backbone by means of hyaluronidase; results consistently show that prolonged laser heating of cartilage induces denaturation/degradation of proteoglycan and can also result in local mineralization of the cartilaginous matrix.

The small channels seen on a section of cartilage irradiated for 3 s may facilitate transport of traces of proteoglycan units split from the hyaluronic backbone (Sobol *et al.*, 1999a). The size of the holes found in cartilage matrix corresponds well with a proteoglycan unit possessing a length of ~ 300 nm–400 nm and a width of ~ 80 nm (Comper, 1991). These results also correspond well with the changes in light scattering behaviour observed in cartilage after laser-induced stress relaxation and reshaping of cartilage occur (Sviridov *et al.*, 1996; Wong *et al.*, 1998a,b): the number of scattering centres first increases due to the short-time liberation of proteoglycan units and then decreases again after the new proteoglycan configuration has being formed. As water transport in cartilage matrix is essential for the survival and function of this tissue (Caplan, 1984), the occurrence of additional channels induced by the laser treatment may also have a positive impact on the healing of diseased cartilage.

The mechanism of stress relaxation

It has been suggested that the underlying mechanism of laser reshaping is mainly connected to a phase transition of cartilaginous bound water to free water taking place at a temperature of $T_w \cong 70°C$ (Sobol *et al.*, 1994, 1997; Sobol, 1995). However, the precise details of this mechanism are still under investigation. It is still an open question as to how the phase transition of the state of water can cause an irreversible stress relaxation. Several possible mechanisms for the laser-induced stress relaxation accompanying the liberation of bound water in cartilage have been considered (Sobol, 1995; Sobol *et al.*, 1997, 1999a): firstly, local mineralization of the tissue caused by neutralization of negatively charged groups of the proteoglycan by positive Na^+ or Ca^{2+} ions without altering either the collagen and proteoglycan microstructure (*Figure 20.7* (a)); secondly, local depolymerization of proteoglycans aggregates under short-time laser heating to temperatures above 70°C followed by a re-formation of the proteoglycan structure without pronounced denaturation (and resulting dramatic structural changes) of the cartilage matrix (*Figure 20.7* (b) and *Figure 20.7* (c)); thirdly, short-time breaking of bonds between the collagen and proteoglycan subsystems facilitating a decrease in internal stress in the cartilage by the alteration of the spatial structure of the proteoglycans; and fourthly, destruction (de-polymerization) of the collagen network.

A better understanding of the precise mechanism of laser-induced stress relaxation in cartilage could possibly be obtained from information on both the degree and the characteristic length scale of local structural alterations that accompany the process of laser reshaping of cartilage. In this regard, Sobol *et al.* (2000a) have recently obtained some indirect evidence of the first two possible mechanisms above on the basis of AFM (*Figure 20.6*) and light scattering techniques measurements. *Figure 20.6* (c) (Sobol *et al.*, 2000a) shows small (sub-micron) crystals arising from CO_2 laser radiation for 30 s. As the EDX analysis has shown, these crystals are sodium carbonate, which, as we have noted above, is a chemically unstable compound and disappears in a few days. Therefore, the local mineralization with sodium carbonate crystals could be responsible for just the short-term stability of laser-treated cartilage.

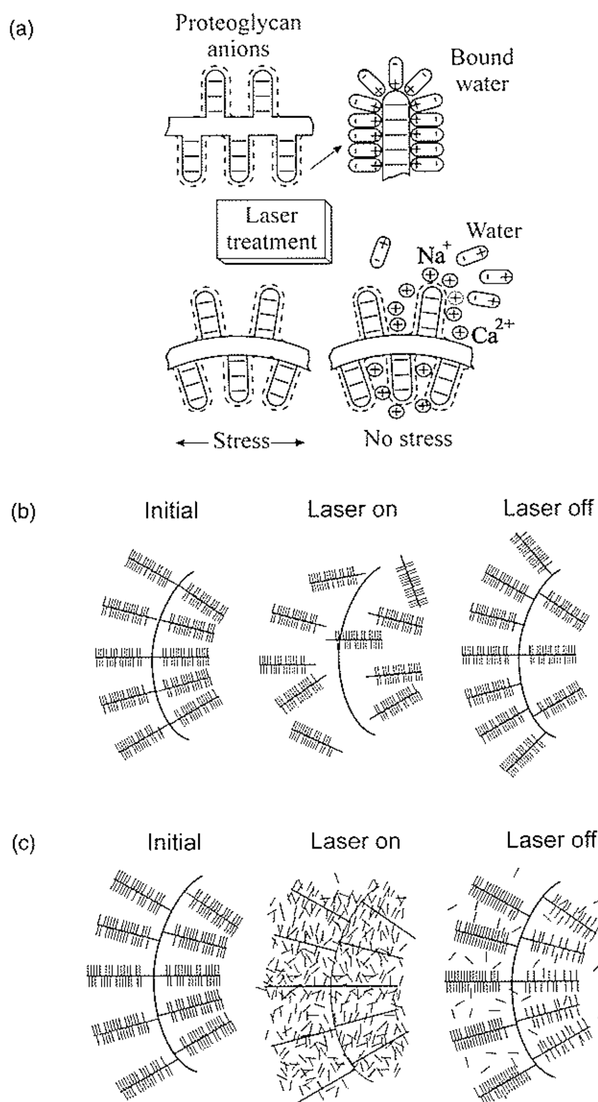


Figure 20.7. Mechanisms of stress relaxation in cartilage: (a) local mineralization; (b) separation of PG units; (c) separation of CS chains (Sobol *et al.*, 1997, 2000a).

Sobol and co-workers have recently studied porcine ear cartilage irradiated *in vivo* (Sobol *et al.*, 2000b) and shown that much more stable crystals (probably calcium carbonate crystals) are evident in the laser treated zone even four months after laser irradiation. On the other hand, comparison of irradiated and non-irradiated cartilage (*Figure 20.6* (a) and (b)) shows that laser radiation may promote the formation of some pores in the ECM. These additional pores could be traces of the PG or CS leaving their sites at the hyaluronic acid or protein core. To test this assertion, those workers carried out the following experiment. Cartilage pieces immersed in buffer were irradiated with a laser in order to study whether cartilage components may

Table 20.1. Effect of laser radiation on molecular weight (weight average, M_w) of chondroitin sulphate (from Jumel *et al.*, 2000)

Sample	Solution temperature (as a result of irradiation)	M_w (g/mol)
Chondroitin sulphate, non-irradiated	Ambient	21,200 ± 4,800
Chondroitin sulphate, irradiated	70° C	17,400 ± 2,400
Chondroitin sulphate, irradiated	90° C	15,500 ± 2,500

diffuse away from the matrix and into the surrounding aqueous medium as a result of laser treatment. Analysis by molecular hydrodynamics using both 'SEC/MALLS' (size exclusion chromatography coupled to multi-angle laser light scattering) and sedimentation velocity measurements in the analytical ultracentrifuge have shown that: (a) laser irradiation decreases the molecular weight (molar mass) of chondroitin sulphate (see *Table 20.1* and Jumel *et al.*, 2000); and (b) laser irradiation of cartilage induces diffusion of macromolecules into the medium. As far as those PGs that cannot diffuse through the ECM, this last result suggests that the mechanism of laser induced stress relaxation could be connected with separation and motion both of PG units and chondroitin sulphate (CS) chains. The molecular aspects of laser effect on cartilage and its component is clearly an area of fruitful study and, in the future, will provide valuable underpinning information to the macroscopic studies.

Water exchange and transport in cartilage

It is well known that the process of water movement in cartilaginous tissue is of great importance, and controls cartilage nutrition. The two different forms of water in cartilage, namely 'free' and 'trapped' (ie exchangeable and non-exchangeable), have been the subject of intense discussion in the past. For example, Maroudas (1970), Maroudas and Schneiderman (1987) concluded that all water in cartilage is exchangeable, whereas Torzilli *et al.* (1982) and Torzilli (1985, 1988) has suggested the existence of a considerable fraction of 'trapped' water that does not participate in water movement through the biological tissue. The mechanism for water diffusion and the role of tissue consolidation in the transport of water through cartilage have been studied by Torzilli and Mow (1976).

A Fourier Transform InfraRed (FTIR) absorption spectrum for cartilage is presented in *Figure 20.8*. It was shown by Bagratashvili *et al.* (1997) that the shape of a spectral line of the first overtone (ca. 5,300 cm^{-1}) of bound water in the cartilage differs both from that of free water and from that of pure distilled water. FTIR spectra of water obtained by Bagratashvili *et al.* (1997) at different times and different temperatures were used to estimate the water content in heated cartilage. It was shown by these workers that the water liberation kinetics could not be described by a simple exponential function of time. For moderate heating temperatures, there are three regimes of water liberation: slow (at the beginning of the heating process), then quick, and finally slow again. The rate of liberation of water is strongly dependent on the sample thickness. The dimensions of cartilage samples decreased in the course of the drying process. The kinetics of water re-absorption in dried cartilage is fully reversible for drying temperatures less than 65°C, and non-reversible for higher temperatures. When cartilage was dried at 340 K for a long period of time (24 hours), and then

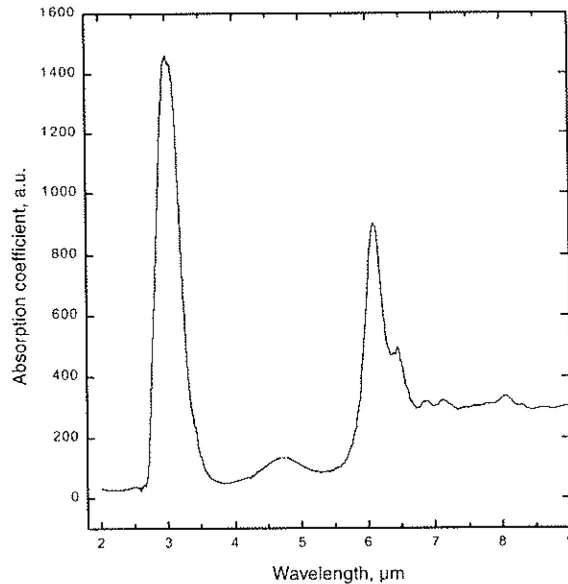


Figure 20.8. Absorption spectra of cartilage (Sobol *et al.*, 1999b).

placed in water, its initial weight value was not re-attained, and a calculation showed that the cartilage did not reabsorb up to 13% of its original water.

Considering the results of calorimetric measurements, it might be concluded that the 70°C maximum corresponds to the bound-to-free phase transition of water in the cartilage which controls cartilage reshaping under laser irradiation (Sobol *et al.*, 1994; Sobol, 1995). The second maximum, around 100°C, corresponds to the energy input required for the evaporation of free water. By measuring the areas under the peaks (approximated as Gaussian shaped curves), it is possible to obtain a value for the ratio $\varepsilon Q_r/Q_e = 0.25$ where Q_e is the heat of evaporation of water and Q_r is the heat of transformation of bound-to-free water in cartilage, assuming $Q_r = (1.5 \pm 0.3)$ kJ/g and $Q_e = 2.3$ kJ/g (Sobol *et al.*, 1996c) where ε is the fraction of bound water and Q_r and Q_e, we find $\varepsilon = (0.04 \pm 0.08)$. Thus, the proportion of bound water in cartilage is around 4%. It is worth noting that this value is less than the proportion of 'trapped' water found by Torzilli *et al.* (1982) and Torzilli (1987) for articular cartilage, which has a different structure, compared to hyaline cartilage used in the experiments of Sobol and co-workers.

Mass transfer of water in the bulk cartilage, as opposed to surface evaporation kinetics appears to be the dominant process (Bagratashvili *et al.*, 1997): this assertion arises from the dependence of the square of the drying time upon the sample thickness. The strong increase observed in the drying rate with temperature also shows that the process is thermally controlled. A value for the activation energy of water diffusion, $U = 1.7$ kJ/g, has been obtained which is consistent with the heat of transformation of bound-to-free water in cartilage, $Q_r = (1.5 \pm 0.3)$ kJ/g (Sobol *et al.*, 1996c). From this it has been deduced that water movement in cartilage is connected with the alternate breakage and formation of bonds between water molecules and PGs. The existence of such proteoglycan 'traps' leads to a decrease in the diffusion

coefficient by an order of magnitude compared to that typical for various organic compounds in free water, or for the self-diffusion of water (Kikoin, 1976). This process is similar to the diffusion and trapping of hydrogen in metal containing pores, micro-cracks and other structural defects (Oriani, 1970). One can speculate that the mechanism of water diffusion in cartilage is adsorption and de-sorption by proteoglycans.

The kinetics of water liberation may be described on the basis of the theory of phase transformation. Assuming that the transformation rate is proportional to the bulk of the remaining initial phase, and to the probability of the liberation of water molecules, Sobol (1995) has given the following relation:

$$d\omega /dt = (1-\omega)\, N\nu\, exp\,(-QM/RT) \qquad (20.1)$$

where ω is the proportion of new phase, N is the density of water molecules bound by PGs, M is the molecular weight of water (18 g/mol), Q is the heat of transformation, and ν is the vibration frequency of bound water molecules. Integrating *Equation 20.1*, we obtain, (for constant N and T)

$$\omega = 1-exp\,(-Kt),\ \text{where}\ K = N\nu\, exp\,(-QM\,/\,RT) \qquad (20.2)$$

Equation 20.2 describes the water liberation kinetics well, when $T < 340$ K. The Q value evaluated by means of *Equation 20.2* is 1.6 kJ/g, which is very close to the activation energy of diffusion.

The study of the kinetics of water absorption by dried cartilage shows that both processes are fully reversible when the drying temperature is less than the temperature of any detectable bound-to-free water transformations ($T < 330$ K). The partial irreversibility of the drying process at higher temperatures may be due to pronounced alterations in PG and collagen structure (Sobol *et al.*, 1994, 1996c). Note that both above QM and Q_rM values are close to the characteristic value of the energy of hydrogen bonds in ice, which is 25 kJ/mol (Whalley, 1976); indeed, they are somewhat higher than that for pure water (16 kJ/mol) and much higher than the typical energy for weak Wan der Waals bonds (from 5 to 10 kJ/mol) (Luck, 1976).

The above phase transition could be related to a denaturation/degradation of the collagen micro-system in cartilage. Luescher *et al.* (1974) has reported the denaturation energy of 12 cal/g (50 J/g, corresponding to the value for intermolecular hydrogen bonds). The important role of water in stabilizing the structure of collagen molecules has been established (Yanas and Tobolsky, 1967). The transformation which occurs when collagen is heated is known to be influenced by the water content of collagen and has been interpreted as a first-order phase transition involving the melting of crystalline regions in the collagen molecules. But, as shown by Bigi *et al.* (1987), water acts as a swelling agent on dried collagen, increasing chain mobility with resultant disorder of the structure.

Chain mobility is decreased in air-dried samples. Calorimetry experiments have shown (Bigi *et al.*, 1987) that there is denaturation peak in air-dried collagen, but this peak disappears when the examination is repeated immediately after denaturation. When the sample is stored in air for two months after denaturation, the maximum appears again at the same temperature (52°C). When the sample is stored in water for an hour, the peak appears again (but at a higher temperature of about 80°C). The enthalpy for the denaturation process is ~40 to 45 J/g, corresponding to a temperature

rise from 51°C (for rehydrated sample) to 112°C (for air-dried sample). So, when water is removed from collagen, its denaturation temperature increases to 112°C, and the chain mobility decreases, which is precisely the opposite of the situation for laser heating of cartilage considered above when water liberation in cartilage allows some segments of the PG structure to move relative other parts of the ECM.

Torzilli (1993) has studied mass transfer of various compounds in ECM and shown that increasing external solute concentration by 100-fold results in no significant change in the rate of mass transport of various molecules (glucose, inulin, dextran) into a cartilage matrix.

Values for the activation energy for both the diffusion of water and also PGs in ECM are close to one another (Sobol *et al.*, 1996c; Jamieson *et al.*, 1987). As shown by Scott (1988), the energy of chondroitin sulphate association is 21 kJ/mol, which is also close to these values. It is therefore difficult to identify the process responsible for the value of the energy of activation Q measured.

Theoretical modelling

The heterogeneous distribution of negatively charged groups in the polymeric proteoglycan chains is the reason for intra-molecular repulsive forces and provides a molecular source of internal stress in cartilage tissue. Stress relaxation in cartilage is due to redistribution of the proteoglycan structure to make it more homogeneous. From this, it could be surmised that, to provide stress relaxation, some parts of the proteoglycan have to reorient sufficiently (ie across a ‘characteristic distance’) to facilitate a redistribution of the charge heterogeneity.

For laser-induced structural transformations in biopolymers, both the local size distribution of diffusing particles and the mechanism of mass transfer are unknown and may change during the process of laser irradiation, thereby making any theoretical analysis of the mass transfer problem extremely difficult. We describe here a simple, yet general, model of mass diffusion in cartilage without identification of a specific geometry, shape or size of the diffusing species. The feasibility of this approach is supported by experimental data (Torzilli, 1993) showing that diffusion in proteoglycan solutions increases with temperature without a significant dependence on size or molecular weight. Additionally, such a model is consistent with the view of mass transfer in cartilage as being a process of successive adsorption and desorption of water by proteoglycans (Bagratashvili *et al.*, 1997). This model illustrates the principle features of laser heating and structural alterations in cartilage and allows examination of various process control parameters and prediction of the condition for laser reshaping and denaturation.

It is known that the water contained within cartilage is of importance for maintaining its structure (Comper, 1991), and, at a certain temperature $T_w \cong 70$°C, the ‘bound’-to-‘free’ transition of cartilaginous water increases mutual mobility of parts of the matrix and may lead to stress relaxation and denaturation (Sobol, 1995; Sobol *et al.*, 1997; Bagratashvili *et al.*, 1997). The dissociation of proteoglycan aggregates and irreversible reorganization of the proteoglycan structure under prolonged heating over 70°C has been documented by Jamieson *et al.* (1987). Liberation of bound water also promotes a weakness of collagen-proteoglycan interaction (Scott, 1988): this may also alter the cartilage structure and result in stress relaxation and/or denaturation.

The theoretical model of laser-induced structural alteration and stress relaxation in cartilage proposed by Sobol *et al.* (1999a) is based on the assumption that laser heating resulting in bound-to-free transition of water allows some parts (segments) of the PG micro-system (PG units or/and CS) to move relative to other parts of the ECM. According to this model, the displacement of these segments only takes place when the temperature rises above 70°C and will result in an irreversible alteration of tissue structure.

When the temperature drops, new bonds arise and new configurations result. So, the degree of structure change depends on the spatially averaged distance of mass transfer. The effective mass transfer by diffusion takes place only during a given period of time, τ_d, during which the temperature is higher than T_w. The purpose of the Sobol *et al.* (1999a) model is to provide a means for calculating the distance (L) of mass diffusion, assuming that the mass transfer takes place only at $T > T_w$. When L is much less than a 'characteristic distance' (see above) of proteoglycan aggregate L_0, the movement of separate elements cannot lead to a dramatic non-reversible structure alteration (denaturation). Denaturation occurs when $L > L_0$. Proteoglycan aggregates could be a few micrometres in size (Comper, 1991). So, a value for $L_0 \cong 5$–$10\ \mu m$ could be chosen as the conventional boundary between two regimes of laser treatment: *denaturation* and *non-destructive* stress relaxation in cartilage. The approach to the heating and mass transfer problems given by (Sobol *et al.*, 1999a) will now be considered. These follow a scheme suggested earlier by Sobol (1995). It is assumed that the diffusion coefficient sharply increases with temperature, and that mass transfer processes do not have a significant affect on laser heating of a tissue.

THE HEAT TRANSFER PROBLEM

Assuming that the laser beam diameter (d) is much larger than the thickness of the cartilage specimen (l), consider the one-dimensional equation of thermal conductivity (Sobol, 1995)

$$\partial T/\partial t = \chi\,(\partial^2 T/\partial x^2) + f(x,t,T) \qquad (20.3)$$

Here $T(x, t)$ is the tissue temperature depending on time (t) and on distance (x) from the irradiated surface,

$$f(x, t, T) = (E\alpha/\rho C)\exp(-\alpha x)\ \vartheta(t) \qquad (20.4)$$

where α is the effective coefficient of light absorption by cartilage depending on laser wavelength, ρ *is* density, C is the heat capacity of cartilage, χ is the thermodiffusivity, E is the laser flux density, and $\vartheta(t)$ is the step-wise function describing a pulse repetition laser radiation (assuming the laser pulse intensity is uniform along a pulse duration τ_p).

The initial and boundary conditions for solving *Equation 20.3* are:

$$T(x, 0) = T_0 = 293 \text{ K is room temperature;}$$

$$(\partial T/\partial x)_{x=0} = (\nu\, Qc_s/\lambda)\ exp\,(-Q/RT_s)$$

$$(\partial T/\partial x)_{x=l} = -(\nu\, Qc_s/\lambda)\ exp\,(-Q/RT_s) \qquad (20.5)$$

The conditions (*Equation 20.5*) represent the caloric effect of water evaporation from

both surfaces of a cartilage slab ($x = 0$; $x = l$) and make solution of the thermal conductivity problem nonlinear. Here: Q is the heat of evaporation of water, R is the gas constant, T_S is the surface temperature, ν is the characteristic frequency of water molecule oscillation, λ is thermal conductivity, $c_S = (cN_A)^{2/3}$ is superficial concentration of water assumed to be constant, c is the water concentration in a tissue, and N_A is Avogadro's number. For small values of t, and for not very high temperatures typical for laser reshaping of cartilage, the amount of evaporated water is small and one can neglect alteration of c in the course of laser irradiation.

MASS TRANSFER PROBLEM

Consider a transfer of proteoglycan units (or some other parts of proteoglycan structure) during period of time τ_d for which tissue temperature T exceeds $T_w = 343$ K. When the mass diffusion coefficient D is constant, diffusion length is approximately $L = (D\tau_d)^{1/2}$. When D is a function of temperature and time, the average length of diffusion may be calculated by means of the relation (Sobol, 1995):

$$L = b\sqrt{\int_0^{\tau} D(t)dt} \tag{20.6}$$

where b is a coefficient depending on the geometry of the diffusion process and on the boundary conditions of the respective mass transfer problem (Sobol, 1995).

Taking into account that diffusion only takes place when $T > T_w$ and that D increases with temperature in accordance with the Arrhenius law (Bagratashvili *et al.*, 1997; Jamieson *et al.*, 1987), it can be written:

$$D(T) = \underline{u}(T - T_w)\, D_0 \exp(-V_p/RT) \tag{20.7}$$

Here $u(x)$ is the unit step function: $u(x) = 1$ at $x > 0$ and $u(x) = 0$ at $x < 0$, V_p is the activation energy for proteoglycan diffusion and D_0 is a pre-exponential factor

The solution of the above heat and mass problems has been described in detail by Sobol *et al.* (1999a). In that description, the temperature field $T(x,t)$ and diffusion length L were given as functions of the following parameters: laser flux (E), exposure time (τ_e), pulse repetition rate (f), cartilage slab thickness (l), and optical absorption coefficient (α). The following characteristics of the cartilage were used for the calculations (all thermo-physical properties of cartilage have been taken as equal to those for water): $\rho C = 4.2$ J/cm^{3}K; $\chi = 1.4 \times 10^{-3}$ cm^2/s, $\nu = 9 \times 10^{11}$ Hz; $\lambda = 3 \times 10^{-3}$ W cm^{-1} K^{-1}; $Q = 44$ kJ/mol; $c = 0.8$ g/cm^3; $V_p = 27$ kJ/mol, $D_0 = 4.8 \times 10^{-3}$ cm^2/s; $\tau_p = 1$ ms; $b = 1$; $T_w = 343$ K (Bagratashvili, 1997; Jamieson *et al.*, 1987).

Sobol *et al.* (1999a) have shown from the results of these calculations that:

(1) Water evaporation from the cartilage surfaces strongly influences the internal temperature field.
(2) There is a temperature maximum T_m at a distance some x_m from the irradiated surface.
(3) The values of T_m and x_m increase with τ_e, f and decrease with α.
(4) The temperature distribution changes with time only during the opening period of laser irradiation, and after a time a quasi-stationary profile is obtained. For thicker samples, the time to reach a quasi-stationary profile temperature is longer.

(5) The quasi-stationary value of the surface temperature (T_s) is reached faster than that for the maximal temperature, T_m. Sobol *et al.* (1999a) interpreted this to mean that attempts to control the laser heating process by measurement of the surface temperature alone does not provide adequate information about the heating level and structural alterations in the bulk cartilage sample.

(6) Temperature oscillations reflect the pulse repetition character of laser radiation. The amplitude of these oscillations increases with laser flux density and always is higher at the front sample surface ($x = 0$) than that at x_m.

(7) When the laser treatment stops, the kinetics of cooling slightly depends on E, f and τ_e.

(8) The period of time, τ_d, when $T > T_w$ is strongly dependent on parameters α, E, f and τ_e.

One of the main parameters of laser treatment is the depth of light absorption, ξ_1, which is inversely proportional to the absorption coefficient α, depending on the laser wavelength λ (see *Figure 20.8*). The values of ξ_1 are 10 μm, 0.3 mm, 3 mm and 1 cm, for λ = 10.6 μm, 2.1 μm, 1.32 μm and 1.06 μm, respectively. Laser-induced stress relaxation and structural alteration occur in the depth ξ which is determined by both parameters ξ_1 and ξ_2 where $\xi_2 = (\chi\tau_e)^{1/2}$ is the 'heating depth'. To reshape cartilage of thickness l, the value of ξ must be comparable with the value of l. If $\xi >> l$, the laser irradiation affects the tissues behind the treated cartilage; if $\xi << l$, only a small part of cartilage is affected by laser radiation. The theory predicts heterogeneity of

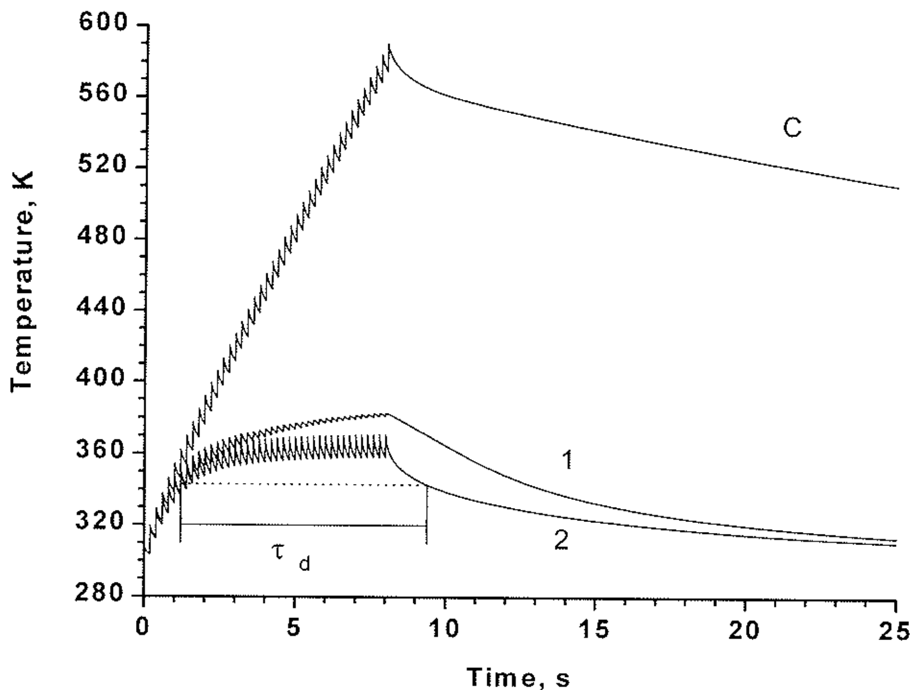


Figure 20.9. Time dependence of the temperature in irradiated cartilage. Calculation, for α = 3 1/cm (Sobol *et al.*, 1999a).

structural alteration and denaturation of the irradiated cartilage. There is a sharp boundary in the bulk of a sample between areas of altered and non-altered structure. *Figure 20.9* (Sobol *et al.*, 1999b) presents the time dependence of temperature in a cartilage slab after heating with 1.32 μm laser radiation (absorption coefficient $\alpha = 3$ 1/cm). It is seen that temperature maximum is located at some distance from the irradiated surface, and the structural alterations could be more pronounced in the bulk of the tissue rather that on its surface. Therefore, histological analysis may well provide different results at different distances from the irradiated surface. From the work of Sobol *et al.* (1999b) there appear *thresholds* of laser-induced structural alteration and denaturation, depending on laser exposure time and wavelength. Depth of structural alteration increases with laser flux density and exposure time. For small E and τ_e, when $L < 5$ μm, alterations of cartilage structure may lead to stress relaxation without tissue denaturation. For higher E and τ_e, mass transfer leads to non-reversible and dramatic changes in structure (tissue denaturation).

An attempt to summarize the experimental conditions for laser reshaping of cartilage when stress relaxation occurs without visible (by means of an optical microscope with a magnification of 300) alterations in cartilage structure has been presented by Sobol *et al.* (1999a) and summarized in *Figure 20.3*. Theoretical values of energy density as a function of exposure time are plotted for various values of diffusion length which could be obtained with the given values for E and τ_e. Small values of the diffusion length ($L \sim$ a few micrometres) represent structural alterations accompanying the laser reshaping of cartilage, whereas the curve with $L > 10$ μm corresponds more to the denaturation (visible changes in structure) of cartilage. The two dashed curves in *Figure 20.3* correspond to the experimental boundaries of the optimal condition region for cartilage reshaping using a holmium laser (Sviridov *et al.*, 1998). Taking into account both the low accuracy of the experimental measurements (Sviridov *et al.*, 1998) and the approximate character of the theoretical model, it is believed that this theory describes well the qualitative picture of the process and also provides a semi-quantitative treatment of the experimental results.

In vivo study of laser reshaping of cartilage and clinical work

A brief but representative potted history of the *in vivo* studies – and the groups of scientists involved – may be reasonably given as follows:

(1) Nasal septum has been removed from the nose, straightened *ex vivo*, irradiated with a CO_2 laser and then auto implanted for three patients. (E. Helidonis, E. Sobol, G. Kavvalos, *Greece*, 1992–1993).
(2) Reshaping of ear cartilage of five rabbits with a CO_2 and a holmium laser (Yu. Ovchinnikov, V. Svistushkin, G. Nikiforova, E. Sobol, A. Sviridov, *Russia*, 1994–1995).
(3) Reshaping of the collapsed tracheal cartilage of three dogs with the 1.44 μm laser radiation (Z. Wang, M.M. Pankratov, D.F. Perrault, S.M. Shapshay, *U.S.A.*, 1995).
(4) Reshaping of ear cartilage for seven pigs with a holmium laser (Yu. Ovchinnikov, V. Svistushkin, G. Nikiforova, E. Sobol, A. Sviridov, V. Bagratashvili, A. Omel'chenko, *Russia*, 1997–1998).

(5) Reshaping of ear cartilage for ten pigs with a holmium laser (N. Jones, E. Sobol, A. Sviridov, A. Omel'chenko, *U.K.*, December 1998).
(6) Laser straightening of the nasal septum for 40 patients with a holmium laser (Yu. Ovchinnikov, V. Svistushkin, A. Shinaev, E. Sobol, A. Sviridov, *Russia,* 1998).

When the laser reshaping procedure was applied to ear cartilage, it was necessary to bypass the skin before the laser light was delivered to the cartilage. Various techniques have been employed to avoid the laser energy being absorbed by skin and to minimize skin damage (Sviridov *et al.*, 1999). The 'minimal skin damage criterion' can be satisfied by what is known as the 'stab incision method', where a small stab incision is made through a guide which has had holes bored at intervals of about 3 mm and arranged in two or three straight parallel rows 3.5 mm apart. Using such a device, Sviridov *et al.* (1999) aimed the laser beam through a 0.6 mm diameter fibre positioned perpendicular to the surface of the cartilage. A few pulses of this were enough to penetrate through the skin using slight pressure. The fibre was then placed close to the surface of the skin and the irradiation procedure continued. In four days, it was difficult to recognize the locations where the incisions had been made. The initial observations from this procedure by Sviridov *et al.* (1999) have been encouraging since the laser treatment produced changes in the shape of the auricular cartilage in every ear examined: it was shown that the auricular cartilage is able to recover its initial shape with time. The degree of stability depends on the cartilage thickness and on the particular parameters of the laser beam used. To find the correct laser conditions, Sviridov *et al.* (1999) have varied the following parameters: laser energy, exposure time, intervals between holes in the skin, the number of treatment lines and treatment interval times.

Most of the experimental conditions examined by Sviridov *et al.* (1999), and in particular, the 'optimal' conditions established *in vitro*, were unfortunately inadequate to obtain a stable configuration of cartilage irradiated *in vivo*. A possible reason for this is that it was not always possible to meet the condition $\xi \cong l$ (see above). When the laser affected zone was much smaller than tissue thickness or laser flux and exposure time were too small, it was not possible to obtain a new stable configuration of cartilage. If the laser flux density and exposure time are too high, some inflammation and necrosis of irradiated tissue can be observed. Sometimes, a new desirable shape of cartilage was obtained, only for it to, unfortunately, subsequently prove unstable.

Sviridov *et al.* (1999) have found an optimum condition for laser reshaping of cartilage of ~ 1 mm in thickness with a holmium laser operating with a pulse repetition rate of 5 Hz: laser flux density of 15 J/cm^2, exposure time 8 s, laser spot diameter of 1 mm with a distance between laser spots of 3 mm. These conditions appear from these studies to permit a new stable configuration of porcine ear to be acquired (see *Figure 20.2*) for at least 3 months. It is important to note that these workers have shown that the above conditions are different from those found *in vitro* (as we have already considered above). Furthermore, histological examination of ear cartilage irradiated with the above regime has shown that new growing cartilage of hyaline type replaces old partly damaged irradiated EXM (*Figure 20.10*). Hyaline cartilage differs from elastic cartilage in that it has no elastic fibres. It appears that hyaline cartilage can be formed as a result of the effect

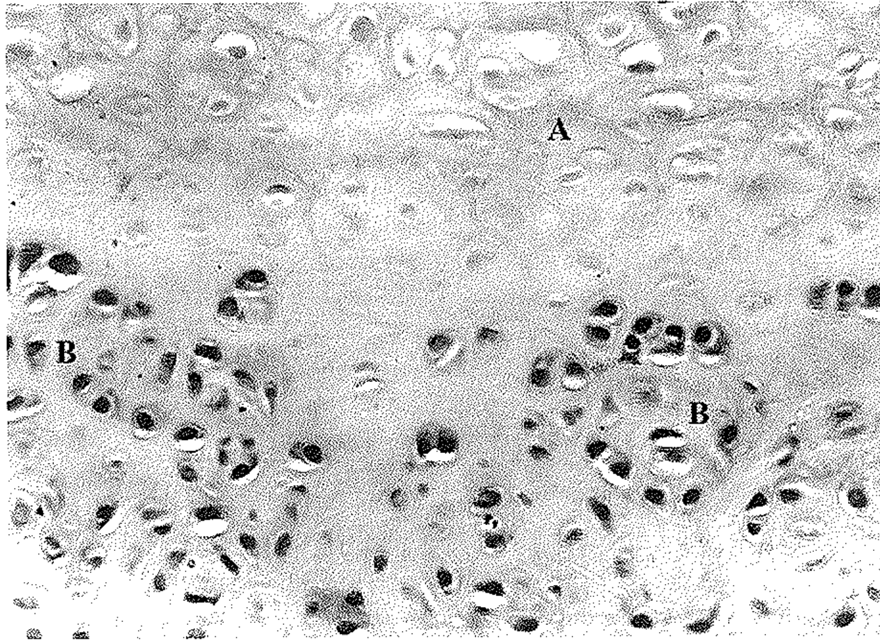


Figure 20.10. Histological examination of cartilage structure. The area of laser treated cartilage in 98 days after operation. A – the necrosis zone, B – regenerated hyaline cartilage – chondrocytes with specific lacunas are seen. Colouring by hematoxylin-eosine, × 400 (Sviridov *et al.*, 1999).

of mechanical stress on chondrocyte function. Sviridov *et al.* (1999) found that, in cases where stable changes in the auricular shape were attained, elastic fibres were destroyed and did not reform in the regenerative zone. It appears that cartilage regeneration occurs more intensely near the area close to where the laser has caused some structural alteration or damage. It is, therefore, possible from these studies to hypothesize that the regeneration of cartilage is probably stimulated by laser-induced stress redistribution, and also by a break of some chemical bonds in the tissue. It is possible that an alteration in porous structure of the ECM may promote tissue regeneration by an improvement in the diffusion of nutrients through the area of altered structure to chondrocytes.

The clinical results of laser reshaping of nasal septum cartilage performed at the Medical Academy of Moscow by Sobol *et al.* (2000b) have shown promising results. Forty patients have been operated on using a holmium laser to correct a deformed cartilage (Sobol *et al.*, 2000b). The laser reshaping is a bloodless, painless procedure, which takes only a few minutes to straighten nasal septum. The stability of the new shape and possible side effects have been examined during a twelve-month period. For 36 of these patients, headaches and other negative symptoms have disappeared within one or two weeks as a result of laser treatment. Examination of the nose before the operation and in six months after the laser irradiation have been shown by these workers to produce pronounced improvement of the airway for 32 patients (see *Figure 20.11*). Correction of the width of the nasal airway of a human nose is shown in *Figure 20.12*. In that study (Sobol *et al.*, 2000b), 4 patients did not show any

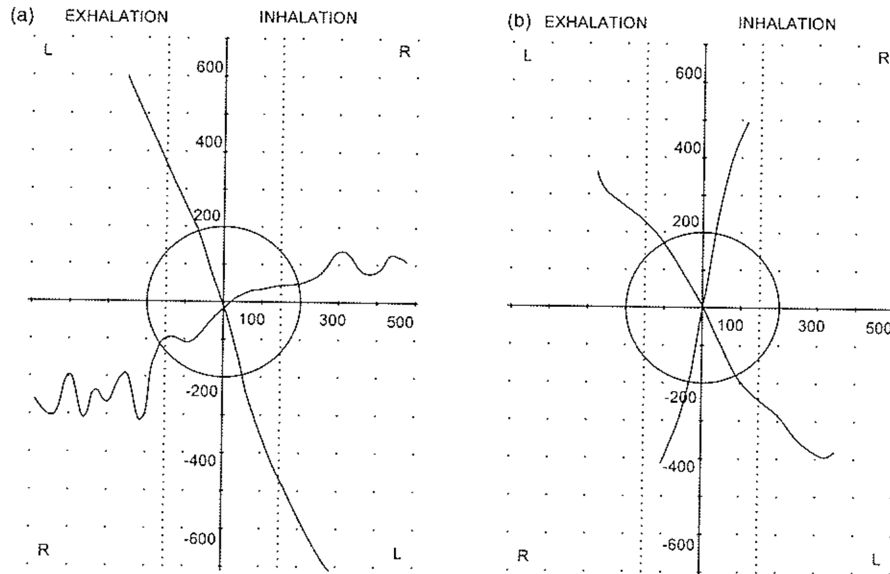
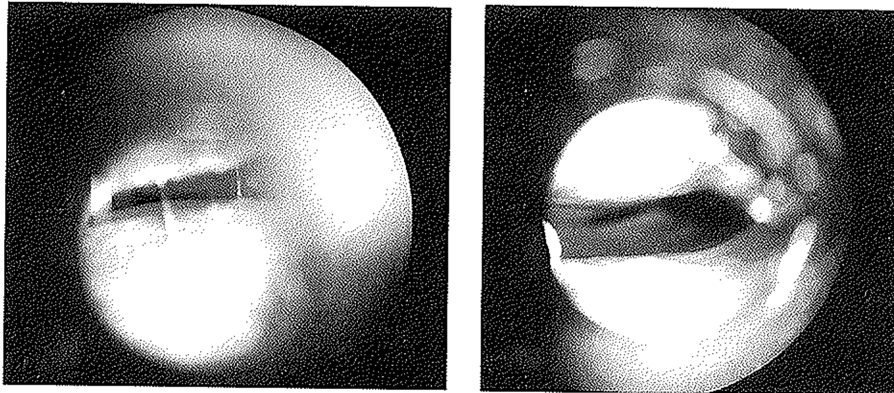


Figure 20.11. Rhino-gasometrical examination of the human nose (a) before and (b) six months after the laser reshaping (Sobol *et al.*, 2000b).

improvement after the first laser reshaping procedure, and have been treated for the second time with a higher laser flux density. Only 2 patients from 40 did not show any improvement. These 2 patients, in fact, had thicker and crested nasal septa, which could not be heated enough by holmium laser irradiation. Further increase of laser flux applied, for one of these patients, led to overheating of the nasal septum in which some holes occurred after a month after the laser treatment. Since the laser method clearly failed for these patients, conventional surgery was resorted to. No secondary effects were observed for all the patients undergoing the laser reshaping procedure.



(a) (b)

Figure 20.12. Correction of the width of the airway of a human nose (a) before, (b) after the laser reshaping of nasal septum (Sobol *et al.*, 2000b).

Conclusions

From the recent studies we have surveyed here, it appears that laser shaping of tissue has a potential use in those types of surgery that involve cartilage, particularly *Otorhinolaryngology*, *Orthopaedics* and *Plastic Surgery*. Its potential applications include: the correction of a bent nasal septum; the treatment of the stenosis of a tracheal cartilage; correction of the shape of the pinna or external ear; the correction of nasal tip cartilage; making cartilage implants in reconstructive and plastic surgery; the correction of deformities in spinal discs; and regeneration of diseased cartilage.

The results of all the *in vivo* studies and clinical examinations performed by various groups of workers appear very promising, although the investigations of this new technique for non-destructive laser treatment of deformed and diseased cartilage are in progress. The advantages of laser radiation over other heat sources derive from the combination of a bulk energy penetration effect and also the short time of heating and simplicity of the delivery of energy to the tissue. The appropriate conditions for laser reshaping clearly depend on the laser wavelength used, and tissue optical properties and thickness. The optimal conditions *in vivo* appear to differ from those *in vitro*, and this is a problem that is currently under investigation.

The following issues thus remain open:

- Why does new growing cartilage sometimes not keep the new configuration obtained as a result of laser treatment, ie is there a 'memory' of its previous shape?
- What are the *secondary* effects on skin and other components of the ECM (in particular, of the elastic fibres)? These need to be fully understood and ways developed to prevent them.
- Why do the conditions for laser reshaping *in vivo* differ dramatically from those *in vitro*? It could be evidence of the importance of the effect of tissue regeneration. We have yet to understand the mechanisms by which lasers affect chondrocyte movements, their multiplication, and their ability to produce new ECM.

The above questions can only really be properly addressed by a multidisciplinary approach involving both molecular and macroscopic study.

Acknowledgements

The authors thank INTAS (Grant 97-1430), and the Russian Foundation of Basic Research (Grant No 99-02-16855) for financial support.

References

BAGRATASHVILI, V.N., SOBOL, E.N., SVIRIDOV, A.P., OMEL'CHENKO, A.I. AND POPOV, V.K. (1997). Thermal and diffusion processes in laser-induced stress relaxation and reshaping of cartilage. *Journal of Biomechanics* **30**, 813–817.

BIGI, A., COJAZZI, G., ROVERY, N. AND KOCH, M.H. (1987). Differential scanning calorimetry and X-ray diffraction study of tendon collagen thermal denaturation. *International Journal of Biological Macromolecules* **9**, 363–367.

CAPLAN, A.I. (1984). Cartilage. *Scientific American* **251**, 84–94.

CHAO, K., WONG, B. AND MILNER, T. (unpublished). Viability of porcine nasal septal cartilage grafts following Nd:YAG (λ = 1.32 μm) Laser Irradiation.

CHIU, J.C., CLIFFORD, T., GREENSPAN, M., NEGRON, F., PRINCENTHAL, R.A. AND CARTER, J.

(1998). Holmium laser thermodiskoplasy through disk shrinkage with tightening effect. In: *Lasers in Surgery and Medicine*, Suppl.10. Abstracts of the 18th Annual Meeting of the American Society for Lasers in Medicine and Surgery, San Diego, April 1998, pp 14–15.

COMPER, W.D. (1991). Physicochemical aspects of cartilage extra-cellular matrix. In: *Cartilage: Molecular Aspects*. Eds. B. Hall and S. Newman, pp 59–96. Boca Raton, U.S.A.: CRC Press.

CUSACK, S. AND LEES, S. (1984). Variation of longitudinal acoustic. *Biopolymers* **23**, 337.

DILLINGHAM, M.F., PRICE, J.M. AND FANTON, G.S. (1993). Holmium laser surgery. *Ortopedics* **16** (5), 563–566.

DONALD, P.J. (1992). Homographic Cartilage in facial implantation. *Facial Plastic Surgery* **8**, 157–175.

HARDING, S.E. (1997). Hydrodynamic Properties of Proteins. In: *Protein Structure. A Practical Approach*. Ed. T.E. Creighton, Chap. 9. Oxford: University Press.

HELIDONIS, E., SOBOL, E.N., KAVVALOS, G., BIZAKIS, J., CHRISTODOULOU, P., VELEGRAKIS, G., SEGAS, J. AND BAGRATASHVILI, V.N.(1993). Laser shaping of composite cartilage grafts, *American Journal of Otolaryngology* **14**, 410–412.

HELIDONIS, E., SOBOL, E.N., VELEGRAKIS, G. AND BIZAKIS, J. (1994). Shaping of Nasal Septal Cartilage with the Carbon Dioxide Laser - Preliminary Report of an Experimental Study. *Lasers in Medical Science* **9**, 51–54.

HELIDONIS, E.M., VOLITAKIS, I., NAUMIDI, E., VELEGRAKIS, G., BIZAKIS, J. AND CHRISTODOULOU, P. (1995). The histology of laser thermo-chondroplasty. *American Journal of Otolaryngology* **15**, 423–428.

JAMIESON, A.M., BLACKWELL, J., REIHANIAN, H., OHNO, H. AND GUPTA, R. (1987). Thermal and solvent stability of proteoglycan aggregates by quasielastic laser light-scattering. *Carbohydrate Research* **160**, 329–341.

JUMEL, K., HARDING, S., SOBOL, E., OMEL'CHENKO, A.I., SVIRIDOV, A. AND JONES, N. (2000). Aspects on the Structural Integrity of Chondroitin Sulphate under non-destructive laser irradiation of cartilage (in preparation).

KIKOIN, I.K. (Ed.) (1976). *Tables of Physical Values*. Atomizdat, Moscow.

LUCK, W.A. (1976). *Hydrogen Bond*. Vol.3. Eds. P. Shuster, G. Zundel and C. Sandorfy. p 1367. Amsterdam, NewYork & Oxford: North-Holland.

LUESCHER, M., RUEGG, M. AND SCHINDLER, P. (1974). Effect of hydration upon the thermal stability of tropocollagen and its dependence on the presence of neutral salts. *Biopolymers* **13**, 2489–2503.

MAROUDAS, A. (1970). Distribution and diffusion of solutes in articular cartilage. *Biophysical Journal* **10**, 365–379.

MAROUDAS, A. AND SCHNEIDERMAN, R. (1987). 'Free' and 'Exchangeable' or 'trapped' and 'non-exchangeable' Water in Cartilage. *Journal of Orthopaedic Research* **5**, 133–138.

MERTIG, M., THIELE, U., BRADT, J., LEIBIGER, G., POMPE, W. AND WENDROCK, H. (1997a). Scanning force microscopy and geometrical analysis of two-dimensional collagen network formation. *Surface and Interface Analysis* **25**, 514–521.

MERTIG, M., SOBOL, E. AND POMPE, W. (1997b). Scanning force microscopy of laser-irradiated cartilage. In: *Contribution to the Annual Conference of the German Physical Society*, Muenster, March 1997.

NASEEF, G., FOSTER, T., TRAUNER, K., SOLHPOUR, S., ANDERSON, R. AND ZARINS, B. (1997). The thermal properties of bovine joint capsule. The basic science of laser- and radiofrequency-induced capsular shrinkage. *American Journal of Sports Medicine* **25**, 670–674.

OMEL'CHENKO, A.I., BAGRATASHVILI, V.N., SOBOL, E.N. AND DMITRIEV, A.K. (1999a). Mechanical examination of cartilage reshaped under laser radiation. *Advanced Materials* **3**, 56–63.

OMEL'CHENKO, A.I., BAGRATASHVILI, N.V., DMITRIEV, A.K. AND SOBOL, E.N. (1999b). Acoustic control of laser shaping of cartilage. In: *Laser Spectrometry and Optical Diagnostics: Novel Trends and Applications in Laser Chemistry, Biophysics, and Biomedicine*. ICONO'98, **3732**, Chap. 49, pp 312–319.

ORIANI, R.A. (1970). The diffusion and trapping of hydrogen in steel. *Acta Metallurgica* **18**, 147–157.

OVCHINNIKOV, YU.M., NIKIFOROVA, G.N., SVISTUSHKIN, V.M., SOBOL, E.N., SVIRIDOV, A.P. AND OMEL'CHENKO, A.I. (1995). Modification of cartilage form in response to laser radiation. *Annals of Otorhinolaryngology* **3**, 5–10.

POOLE, A.R. AND ROSENBERG, L.C. (1987). Proteoglycans, Chondrocalcin, and the Calcification of Cartilage Matrix in Endochondral Ossification. In: *Biology of Proteglycans*. Eds. T.N. Wight and R.P. Mecham, pp 187–210. San Diego, U.S.A.: Academic Press.

PULLIN, J.G., COLLIER, M.A., PRAJNAN, D.V.M., SMITH, R.L., DEBAULT, L.E., JOHNSON, L.L. AND WALLS, R.C. (1996). Effects of Holmium:YAG laser energy on cartilage metabolism, healing, and biochemical properties of lesional and perilesional tissue in a weight-bearing model. *Arthroscopy* **12** (1), 15–25.

SATO, M., ISHIHARA, M., ARAI, T., ASAZUMA, T., KIKUCHI, T., KIKUCHI, M. AND FUJIKAWA, K. (1999). A study for the optimum Ho:YAG laser irradiation condition on the percutaneous laser disc decompression (PLDD): In vitro quantitative evaluation of the influence on disc cells using three-dimensional cell culture system. *Society of Photo-Optical Instrumentation Engineers. Proceedings. Laser-Tissue Interaction X: Photochemical, Photothermal, And Photomechanical* **3601**, 387–391.

SCOTT, J. (1988). Proteoglycan-fibrillar collagen interaction. *Biochemical Journal* **252**, 313–323.

SOBOL, E.N. (1995). *Phase Transformations and Ablation in Laser-Treated Solids*. pp 316–322. New York, U.S.A.: John Wiley & Sons, Inc.

SOBOL, E.N., BAGRATASHVILI, V.N., SVIRIDOV, A.P., OVCHINNIKOV, YU.M., SHECHTER, A.B., NIKIFOROVA, G.N. AND SVISTUSHKIN, V.M (1994). Cartilage shaping under laser radiation. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **2128**, 43–49.

SOBOL, E.N., BAGRATASHVILI, V.N., SVIRIDOV, A.P., OMEL'CHENKO, A.I., SHECHTER, A.B., ISAEV, M.I. AND ZENGER, V.G. (1996a). Study of Cartilage Reshaping with a Holmium Laser. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **2623**, 544–547.

SOBOL, E.N., BAGRATASHVILI, V.N., SVIRIDOV, A.P., OMEL'CHENKO, A.I., SHECHTER, A.B., HOWDLE, S. AND HELIDONIS, E.(1996b). Phenomenon of cartilage shaping using moderate laser heating. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **2623**, 548–553.

SOBOL, E.N., BAGRATASHVILI, V.N., SVIRIDOV, A.P., OMEL'CHENKO, A.I., SHECHTER, A.B., DOWNES, S., HOWDLE, S. AND JONES, N. (1996c). Stress relaxation and cartilage shaping under laser radiation. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **2681**, 358–363.

SOBOL, E., SVIRIDOV, A., BAGRATASHVILI, V., OMEL'CHENKO, A., KITAI, M. AND POPOV, V. (1997). Mechanism of laser-induced stress relaxation in cartilage. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **2975**, 310–315.

SOBOL, E., KITAI, M., JONES, N., SVIRIDOV, A., MILNER, T. AND WONG, B. (1999a). Heating and structure alterations in cartilage under laser radiation with regard of water evaporation. *Quantum Electronics* **35**, 532–538.

SOBOL, E., SVIRIDOV, A., KITAI, M., EDWARDS, G., GILLIGAN, J. AND TOLK, N.H. (1999b). Effect of wavelength on threshold and kinetics of tissue denaturation under laser radiation. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **3601**, 122–129.

SOBOL, E.N., OMEL'CHENKO, A.I., MERTIG, M. AND POMPE, W. (2000a). Scanning force microscopy of the fine structure of cartilage irradiated with a CO_2 laser. *Lasers in Medical Science* **15**, 15–23.

SOBOL, E., SVIRIDOV, A., BAGRATASHVILI, V., OMEL'CHENKO, A., OVCHINNIKOV, YU.M., SHECHTER, A.B., SVISTUSHKIN, V. AND SHINAEV, A. (2000b). Laser reshaping of nasal septum cartilage. Clinical results for 40 patients. *International Symposium on Biomedical Optics*, 22–28 January, 2000, San Jose. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **3907** (to be published).

SVIRIDOV, A., SOBOL, E., BAGRATASHVILI, N., OMEL'CHENKO, A., JONES, N. AND LOWE, J. (1996). Dynamics of Optical and Mechanical Properties of Cartilage at Laser Heating. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **2923**, 114–117.

SVIRIDOV, A.P., SOBOL, E.N., JONES, N. AND LOWE, J. (1998). Effect of holmium laser radiation on stress, temperature and structure in cartilage. *Lasers in Medical Science* **13**, 73–78.

SVIRIDOV, A.P., SOBOL, E.N., BAGRATASHVILI, V., OMEL'CHENKO, A., OVCHINNIKOV, YU. M., SHECHTER, A.B., SVISTUSHKIN, V.M., NIKIFOROVA, G.N. AND JONES, N. (1999). *In vivo* study and histological examination of laser reshaping of cartilage. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **3590**, 222–228.

TORZILLI, P.A. (1985). Influence of cartilage conformation on its equilibrium water partition. *Journal of Orthopaedic Research* **3**, 473–483.

TORZILLI, P.A. (1988). Water Content and Equilibrium Water Partition in Immature Cartilage. *Journal of Orthopaedic Research* **6**, 766–769.

TORZILLI, P.A. (1993). Effect of temperature, concentration and articular surface removal on transient solute diffusion in articular cartilage. *Medical & Biological Engineering & Computing* **31**, S93–S98.

TORZILLI, P.A. AND MOW, V.C. (1976). On the fundamental fluid transport mechanisms through normal and pathological articular cartilage during function. II. The analysis, solution and conclusions. *Journal of Biomechanics* **9**, 587–606.

TORZILLI, P.A., ROSE, D.E. AND DETHMERS, D.A. (1982). Equilibrium water partition in articular cartilage. *Biorheology* **19**, 519–537.

VANGSNESS, C.T. AND CHADEN, B. (1993). A Literature Review of Lasers and Articular Cartilage. *Orthopaedics* **16**, 593–598.

VELEGRAKIS, G., VOLITAKIS, M., NAUMIDI, I., BIZAKIS, J., CHRISTODOULOU, P. AND HELIDONIS, E. (1994). Thermochondroplasty of Rabbit Ear Cartilage using the Carbon Dioxide Laser. *Lasers in Medical Science* **9**, 265–272.

WANG, Z., PANKRATOV, M.M., PERRAULT, D.F. AND SHAPSHAY, S.M. (1995). Laser-assisted cartilage reshaping: *in vitro* and *in vivo* animal studies. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **2395**, 296–302.

WANG, Z., PANKRATOV, M.M., PERRAULT, D.F. AND SHAPSHAY, S.M. (1996). Laser-assisted reshaping of collapsed tracheal cartilage. A laboratory study. *Annals of Otology, Rhinology and Laryngology* **105**, 176–181.

WHALLEY, E. (1976). *Hydrogen Bond*. Eds. P. Shuster, G. Zundel and C. Sandorfy. Vol.3, p 1425. Amsterdam, New York & Oxford: North-Holland.

WONG, B., MILNER, T., ANVARI, B., SVIRIDOV, A., OMEL'CHENKO, A., BAGRATASHVILI, V., SOBOL, E. AND NELSON, J.S. (1997). Thermooptical Response of Cartilage During Feedback-Controlled Laser-Assisted Reshaping. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **2970**, 380–391.

WONG, B., MILNER, T., ANVARI, B., SVIRIDOV, A., OMEL'CHENKO, A., BAGRATASHVILI, V., SOBOL, E. AND NELSON, J.S. (1998a). Measurement of Radiometric Surface Temperature and Integrated Backscattered Light Intensity During Feedback-Controlled Laser-Assisted Cartilage Reshaping. *Lasers in Medical Science* **13**, 66–72.

WONG, B., MILNER, T., KIM, H., NELSON, J. AND SOBOL, E. (1998b). Stress Relaxation of Porcine Septal Cartilage During Nd:YAG Laser Irradiation: Mechanical, Optical, and Thermal Responses. *Journal of Biomedical Optics* **3** (4), 409–414.

WONG, B., MILNER, T., SOBOL, E. AND NELSON, S. (1998c). Critical temperature transitions in laser-mediated cartilage reshaping. *Society of Photo-Optical Instrumentation Engineers. Proceedings* **3245**, 161–172.

WONG, B., MILNER, T., KIM, H., TELENKOV, S.A., CHEW, C.F., SOBOL, E. AND NELSON, J. (1999). Characterization of temperature-dependent biophysical properties during laser mediated cartilage reshaping. *Selected Topics in Quantum Electronics* **5**, 1095–1102.

YANAS, I.V. AND TOBOLSKY, A.V. (1967). Crosslinking of gelatin by dehydration. *Nature* **215**, 509–510.