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Abstract. Laser radiation provides a means to control the fields of temperature and thermo mechanical stress, mass transfer, and modification of fine structure of the cartilage matrix. The aim of this outlook paper is to review physical and biological aspects of laser-induced regeneration of cartilage and to discuss the possibilities and prospects of its clinical applications. The problems and the pathways of tissue regeneration, the types and features of cartilage will be introduced first. Then we will review various actual and prospective approaches for cartilage repair; consider possible mechanisms of laser-induced regeneration. Finally, we present the results in laser regeneration of joints and spine disks cartilages and discuss some future applications of lasers in regenerative medicine. [DOI: 10.1117/1.3614565]

Keywords: laser; medical physics; cartilage; regeneration; tissue structure; orthopedics.

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1 Introduction

Cartilage is a kind of highly specialized connective tissue. The structural variety of the cartilage provides its unique biomechanical capacity to bear different kinds of static and dynamic loads over a wide range of intensity. Biological role of cartilage structures stems from their critical significance for growth and development as well as for all kinds of body movements. The exceptional importance of cartilage elements for individual survival is, probably, due to mechanisms of natural selection, resulting in limited reparative potential of this tissue. Scanty cellular sources and low metabolic rate along with avascularity of cartilage contribute to its decreased regeneration ability. As a result of these strong limitations, the injuries of cartilage caused by inflammation, traumas, degeneration, and aging usually become chronic and recalcitrant to any kind of medical treatment. In the USA, according to tentative estimations, the prevalence of all forms of arthritis has been calculated in order of 40 million people; and the annual medical care costs were about 65 billion USD.[1] Degenerative spine diseases are a major cause of back pain that deteriorates the quality of life of patients and often leads to disability. Direct and indirect medical expenses are estimated as more than 90 billion per year.[2]

High prevalence and incidence, as well as the social and economic significance of cartilage pathology, attract great interest to this problem. Considerable efforts have been devoted to study various approaches to restore cartilage structures and to stimulate intrinsic capabilities of the tissue to regeneration. There are several treatment modalities of cartilage restoration suggested for clinical use (see Ref. 3 and referred literature): 1. surgical techniques; 2. controllable cell delivery to the lesion; and 3. tissue engineering applications of biodegradable materials (scaffolds) with cell-seeding and modification of cartilage reparative response by different growth factors and cytokines. Although there is a wealth of information regarding the substitution of lost cartilage by the mentioned approaches, the problem of cartilage repair is still unsolved. The long term results show no completed cartilage regeneration; in many cases, the new growing tissue materially differs from the well organized original cartilage. The reasons of insufficient cartilage reparation are connected with its structural and functional organization and with the difficulties of the precise control of the external physical and chemical effects.[4,5] Regeneration of cartilage may be realized in accordance with the natural genetic program of the cells. The efficacy of any approach aimed to control the regeneration process depends on the solution of three tasks: 1. the ability to reproduce the normal cell differentiation sequence from the progenitor cells to mature chondrocytes, 2. stimulation of the specific subpopulations of the resident cells to proliferation and/or new matrix production, and (c) achievement of adequate spatial organization of the new growing tissue. Probably, the most important feature of the laser-based treatment is the involvement and activation of the intrinsic mechanisms of cartilage repair. Many papers are devoted to the effect of low-intensive lasers on cartilage functional state and reparative ability. However, the effectiveness, as well as the placebo-versus-treatment ratio for low level laser therapy, is still under considerable dispute. A more detailed discussion of this issue may be found elsewhere.[6] This paper is mainly limited with a consideration of the effect of nonablative laser radiation on the cartilaginous cells through their matrix microenvironment to provide natural and optimal conditions for regeneration. Wide ranges of wavelengths, precise localization of the irradiated area, and temporal and spatial modulation of laser radiation are the main advantages of the laser technologies, which may result in specific tissue response. In particular, the laser-induced modification of the cartilage extracellular matrix (ECM) seems to be of great significance in view of some new data on the developmental roles of the matrix molecules and mechanical loads. Although the evidence of

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laser irradiation morphogenetic effects is still largely circumstantial, we consider the available observations to address some possible perspectives of the controlled regeneration of cartilage using nonablative laser treatment. So, the aim of this paper is to review physical and biological aspects of laser-induced regeneration of cartilage, to discuss the possibilities and prospects of its clinical applications. The problems and the ways of tissue regeneration and the types and features of cartilage will be introduced first. Then we will review various actual and prospective approaches to cartilage repair, consider possible mechanisms of laser-induced regeneration, present the results in laser regeneration of joints and spine disks cartilages, and finally, discuss some future medical applications of laser regeneration.

2 Cartilage as a Subject of Regeneration

There are a number of detailed reviews describing the structure and vital functions of cartilages.[3,7,8] The main components of cartilage are cells (chondrocytes) and ECM consisting of water (70 to 80%), collagens, proteoglycans (PGs), hyaluronic acid (HA), and glycoproteins (GP). The PGs consist of glycosaminoglycanes (chondroitin sulphate and keratan sulphate) linked to the core-protein, which, in turn, is bound with HA threads interweaving between collagen fibrils (Fig. 1). PGs have a lot of negative charged groups; and the electrical neutrality of cartilage is due to the presence of positive ions (K^+, Na^+, H^+, Ca^{2+}, Mg^{2+}). There are three types of cartilage tissue: hyaline cartilage (costal, nasal septum, articular cartilage of the joints), fibrous cartilage (annulus fibrosis of the spine disks, Eustachian tube), and elastic cartilage (auricle, epiglottis). Hyaline cartilage first forms in embryos and later transforms into other types of cartilage and bone tissues. The distinguishing features of the ECM of hyaline cartilage are having a very high content of glycosaminoglycanes and the prevalence of collagen type II fibrils.[9–13] Fibrous cartilage is characterized by predominance of collagen type I.[14–16] Matrix of the elastic cartilage possesses elastic fibers. Nasal and some other cartilages are covered with a perichondrium playing an important role in nutrition and growth of the avascular tissue. Articular cartilage has no perichondrium; it gets nutrition from synovial liquid and subchondral bone. An articular cartilage surface is covered by a cell-free *lamina splendens* (LS) consisting mainly of the HA and phospholipids.[17] An important structural and metabolic unit of articular cartilage is a chondron.[11] It includes a chondrocyte and its pericellular matrix (PM) bordered with a pericellular capsule (PC). The chondron is surrounded by territorial and interterritorial matrices. The chondrons and their matrix environment have different mechanical properties.[3,11] The PM is enriched with HA, sulphated PGs, biglycan, and GPs, including link protein and laminin. The PC is predominantly composed of compact thin fibrils of collagen type VI and fibronectin. It is suggested that the PM and PC provide hydrodynamic protection for the chondrocyte against pressure loading and take a part in control of spatial and temporal distribution of newly synthesized macromolecules as well as in the cell-matrix interaction.[11] Territorial and interterritorial matrices are characterized by different degrees of the PGs maturity and with a different proportion of the chondroitin sulphate and keratan sulphate. The heteropolymeric fibrils of collagen types II, IX, and XI (HCF) emerging in the territorial matrice become the major load-bearing element in the interterritorial matrice.[16] These fibrils are in charge of the tissue protection against multidirectional tensions.

A number of molecules that possess signal roles in morphogenetic processes, including chondrogenesis from embryonic development to regeneration, may interact with the receptors of the cellular membrane of chondrocyte. Binding of such morphogenes to the membrane receptors triggers various intracellular signaling cascades to result in regulation of the expression of genes. Hydrostatic pressures and fluid flows as well as multidirectional tensions contribute to tissue water displacement leading to changes of local concentrations of ions and morphogens. The GP molecules (integrins, fibronectin, laminin, etc.) distributing over the ECM serve as important mediators of the signaling molecules. They play an important role in the cell-matrix interactions and operate on the growth of cartilage tissue.

Hyaline cartilage has a zonal structure:[11,18,19] the superficial layer contains fibroblast-like chondrocytes of type I. It is characterized by a decreased level of the PG aggregates (aggrecanes) and by a high content of small leucine-rich PGs (decorin and biglycan). The cells in the middle layer are chondrocytes of type II. They form multicellular clones and keep a certain ability of proliferation. A smaller subpopulation of the middle layer cells is presented by the chondrocytes type III covered with lacunas. These nonproliferating cells are also presented in the deep layer of cartilage. Type IV cells belong to a degrading cell group. Chondrocytes synthesize and degrade all components of cartilage matrix through specialized enzymes (prolyl hydroxylase, lysil oxidase, collagenases, aggrecanases etc.).[16,20] Metabolic activity of the chondrocytes in cartilage is controlled by hormones, various cytokines, growth factors, and vitamins (A, C, and D).[21–24] Ultimately, the biosynthetic and catabolic activities of cartilage cells, as well as the kinetics of the cellular population are governed by the local concentrations of the humoral and insoluble morphogens near the external membranes of chondrocytes.

The main mechanism of cartilage nutrition is diffusion of water carrying low-molecular substances (ions, glucose, amino acids, etc.). As the chondrocytes kinetics are under conditions of hypoxia, their metabolism is generally realized by the anaerobic glycolysis pathway. That, in combination with the chondrocytes paucity, determines a low level of cartilage metabolism. Half life period is three or four years for aggrecans, and about 10 years for collagen.[25] All types of cartilage, especially articular cartage and intervertebral disks, have low repair potential. There is a lot of literature on this topic.[26–37] Extra-articular cartilage is usually repaired by the means of proliferation and chondrogenic differentiation of the perichondrial cells. The defects of hyaline cartilage and the extensive defects of costal and auricular cartilages are usually filled up with fibrous connective tissue or fibrous cartilage, which both do not have adequate functional properties; that determines persistent attempts to find new possibilities for cartilage regeneration. The healing of cartilage defects can be improved with mechanical stimulation, intra-articular application of HA, hormone therapy,[38–41] and also with the use of osteochondral or cartilaginous implants, in particular together with cultivated chondrocytes.[42,43] One of the current leading approaches is *in vitro* growth of the tissue engineering constructs followed with their implantation into cartilage lesion. Autologous chondrocyte implantation (ACI) resulted in the formation

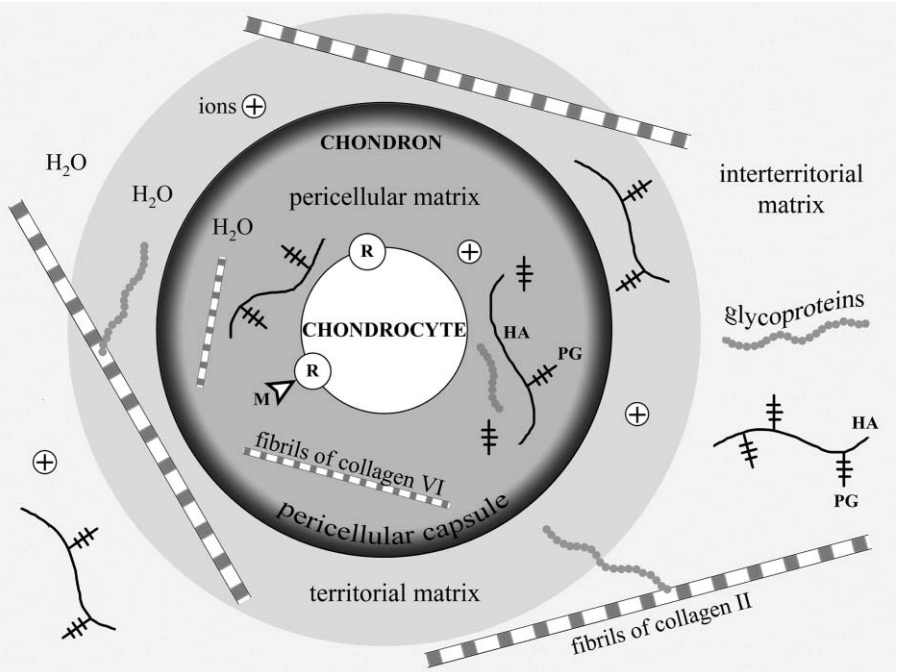

Fig. 1 Cartilage components and structure. PG –proteolycanes; HA – hyaluronic acid, GP – glycoproteins; M – morphogenes; R -molecular receptors of chondrocyte's membrane; (+) ions (K^+, Na^+, H^+, Ca^{2+}, Mg^{2+}).

of hyaline-like tissue with a quite stable clinical outcome.[44,45] But according to the histological data, only 39% of the defects treated with ACI were filled with hyaline cartilage, while 43% were filled with fibrocartilage, and 18% did not show any healing response at all.[46]

Regeneration process is associated with embryonic chondrogenesis mechanisms and partial dedifferentiation of mature cells. Figure 2 shows possible pathways of regeneration-related dedifferentiation of the cells in cartilage. Mesenchymal stem cells (MSC) can differentiate into cartilage cells of various types, including immature and mature chondrocytes, and notochordal and chondrocyte-like cells of the intervertebral disks. These processes are under multilevel control of signaling molecules and mechanical factors. Our main hypothesis is that differentiation and dedifferentiation of cartilage cells, as well as their metabolic activity, may be controlled by direct action of laser radiation on the cells and through laser-induced modification of the ECM.

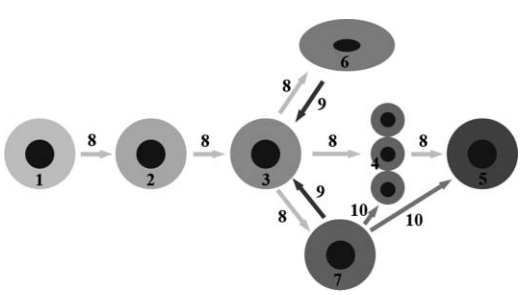

Fig. 2 Differentiation of cartilage cells and possible pathways of their regeneration-related dedifferentiation. 1 – MSC, 2 – pre-chondrocytes, 3 – early chondrocytes (chondroblasts), 4 – columnar chondrocytes, 5 – hypertrophic chondrocytes, 6 – chondrocytes of fibrous cartilage, 7 – chondrocytes of hyaline cartilage, 8 – differentiation pathways, 9 – pathways of limited dedifferentiation, 10 – additional pathways of cellular differentiation (following the enchondral osteogenesis differentiation mechanism).

3 Targets for Laser Effect. Possible Types of Cartilage Response on Laser Radiation

To discuss possible ways of using lasers for cartilage regeneration, it is important to know what effect laser parameters have on (a) different types of the cells; (b) different components of the ECM; (c) signaling molecules produced by the cells and accumulated in the ECM; (d) intercellular and cell-matrix interactions; (e) differentiation and dedifferentiation of the cells, their migration and biosynthesis activity. Feasible pathways promoting cartilage regeneration include: 1. additional cellular supply from bone marrow and blood; 2. biosynthesis amplification of the ECM components, 3. stimulation of the motility of mature chondrocytes, and 4. activation of resident adult stem cells toward their proliferation, differentiation, and ECM production. The main reasons of the low regeneration potential of cartilage are advanced differentiation of the resident chondrocytes and relatively slow metabolism of the tissue. The nonablative laser radiation may provide controllable thermal and mechanical effects (as on the cells, as on the matrix) resulting in activation of the cellular biosynthesis. In particular, nonuniform laser heating of cartilage induces heterogeneous thermal expansion, stress, and also the movement of the interstitial water and ions (see Fig. 3 and Sec. 4).

One of the major obstacles for regeneration of cartilage, including partial-thickness defect of articular cartilage, is its avascularity, which hampers the progenitor cell movement from the blood and marrow to the damaged areas of the tissue. Preventing an entry of unspecialized cells and diminishing the rate of cartilage repair that slow regeneration, nevertheless, may have

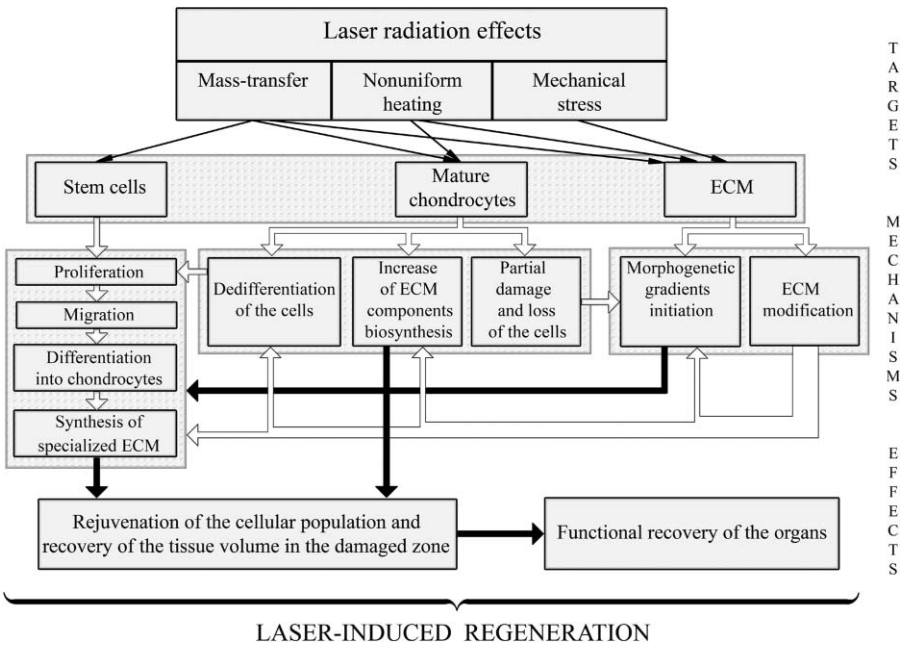

Fig. 3 Targets and mechanisms of the laser-induced regeneration of cartilage. Thin straight arrows show the direct laser influence on the components of cartilage. White thick arrows represent the most important biological responses to laser action. Dark thick arrows show the processes leading directly to regeneration.

its good point, as it may potentially result in the growth of well organized tissue of the hyaline cartilage. Rapid repair of the full-thickness cartilage defects usually leads to undesirable growth of fibrous connective tissue or fibrous cartilage due to the impact of blood and bone-marrow-derived cells. It can be better understood by the following analogy. It is known that skin wound healing resulting in a fibrous scar is going through emergency regeneration due to swift proliferation of unspecialized fibroblasts. Their sources are the precursor cells coming into the wound via blood. These cells have nonspecific genetic program and form scar.[47] In a similar manner, the bone-marrow cells coming to the full-thickness defect of articular cartilage differentiate into the fibroblasts of nonspecific connective tissue or into the chondrocytes of the fibrous cartilage. This provides quick filling of the defect, but fails in functionality of the novel tissue. One of the possible ways to promote growth of the hyaline cartilage in the full-thickness defects of articular cartilage plates can be laser-induced coagulation of the bottom of the defect. This may prevent access of unspecialized precursor cells from the blood or bone-marrow in order to develop more specific, i.e., hyaline cartilage.

It is known that in the course of embryogenesis, the hyaline cartilage forms in the zones undergoing compression load (articular cartilage), whereas, the fibrous cartilage (meniscus, annulus fibrosis of the intervertebral disk) usually develops in the stretched or torsioned zones. Spatial and temporal modulation of laser radiation allows controlling the actual distribution of stretched and compressed zones in cartilage. The mechanical loads are important factors governing an orchestra of chondrogenesis, including the processes of cellular differentiation. Therefore, the nonablative laser treatment may play a triggering role in the differentiation of immature cartilage cells. Laser radiation may probably be responsible for the reverse process of dedifferentiation of the mature chondrocytes leading to the recovery of their ability to divide. Existing natural pathways of cells dedifferentiation (see Fig. 2) open possibilities for tissue correction, in particular, replacement of abnormally grown fibrous tissue by hyaline cartilage possessing adequate mechanical and functional properties (Fig. 3).

Laser radiation can also be used to stimulate proliferation and acquiring the specialized phenotype by resident stem cells or MSC coming through synovial liquid in order to promote their transformation into mature hyaline-like chondrocytes. This approach is critically significant for healing of the partial-thickness defects of articular cartilage. At the same time, as the cellular population in full-thickness cartilage defect is highly heterogenic, laser irradiation may effect the proliferation of different kinds of cells. Thereafter, the additional controlling factor of the ECM architecture should be taken into account. Laser modification of the fine structure of ECM does not change its general organization. This provides natural environments for chondrocytes and promotes restoration of the hyaline type cartilage. One of the important factors is cell movement velocity, which correlates with the alignment of the matrix fibrillar components.[48,49] Nonablative laser irradiation allows structure modification and diffusion properties of ECM. This may support cell movement and favor tissue regeneration.

Laser-induced growth of hyaline cartilage in elastic cartilage was established in the course of *in vivo* experiments on laser reshaping of porcine ears.[50] The effects of laser irradiation on gene expression of chondrocytes and collagen of ECM have been studied for rabbit septal cartilage using laser settings typical for laser reshaping procedure.[51] It was shown that laser irradiation of cartilage does not result in the detection of collagen type I. Only collagen type II was observed after laser irradiation in the corresponding cell culture *in vitro*. This fact indicates that cartilage cellular response to nonablative laser irradiation differs from the reaction of conventional wound healing. Laser irradiation of

cartilage can leave intact collagen and preserve general matrix architecture, which favors chondrocyte survival and promotes new tissue growth. Evidence of hyaline cartilage development in laser-irradiated intervertebral disks was revealed in the animal experiments (see Sec. 5.2). The advantage of the laser effect on chondrocytes proliferation compared to other thermal, mechanical, and chemical effects was demonstrated in Ref. 52. No evidence of chondrocyte DNA replication was observed in tissues heated using nonlaser methods, grown in TGF-β-contained media, or mechanically traumatized. In contrast, for laser irradiated chondrocytes, flow cytometry provided evidence that laser irradiation causes a proliferative response in chondrocytes.

4 Characteristic Features and Physical Mechanisms of Laser-induced Regeneration of Cartilage

Let us consider an indirect laser effect on the cartilaginous cells through ECM. There are some reasons to think that this approach will allow better control structure and properties of the newly growing tissue. Regulable laser effect on mechanical stress and structure of cartilage matrix may lead to a relatively slow regeneration process resulting in the growth of specifically organized tissue, i.e., the cartilage of hyaline type. The interplay of chondrocytes and their microenvironment is of great importance. The majority of cartilage metabolic processes is controlled with mass transfer of tissue water and solved substances. The intensity of water transfer in cartilage has been studied and estimated on the basis of the theoretical models of the pore system with an effective pore size resulting in equal water transfer.[53] The effect of laser radiation on water mass transfer through cartilage has been studied in Ref. 54. An alteration in the diffusion coefficient was attributed to alterations of the pore size in cartilaginous matrix.

Pore formation under laser radiation is one of the known mechanisms of stress relaxation in solids.[55] The difference of the living tissue is that pores can be not only fixed holes, but temporal ways in the cartilage matrix allowing water mass transfer. The term "pore" is used here for an ECM area of enhanced water diffusion that can be permanent or transient, continuity flaw, or cavity filled with gas and/or liquid. The "blocking" of the micro pores system may result in insufficient feeding of chondrocytes followed by various cartilage diseases.[55] One of the essential mechanisms of laser-induced cartilage regeneration is pore "unblocking;" their rearrangement and formation of new pores due to thermo mechanical effect of laser radiation.[56]

Nonuniform heating of the tissue under spatial and temporal modulated laser radiation leads to heterogeneous deformation and stress, which may induce development of gas bubbles and the pores in ESM.[57] The theoretical model based on analysis of the heat activated chemical bonds breaking, describes stress relaxation due to formation of the pores in cartilage matrix under thermal/mechanical load.[58] This model takes into account that the potential barrier for the bond breaking depends on external mechanical stress. The pore formation in cartilage under mechanical load and laser heating was also experimentally studied.[55,56,59] It was shown that laser heating increased both a number of pores and their size in a controllable manner (Fig. 4). These pores intensified water mass transfer in the ESM and improved the feeding of the cell.

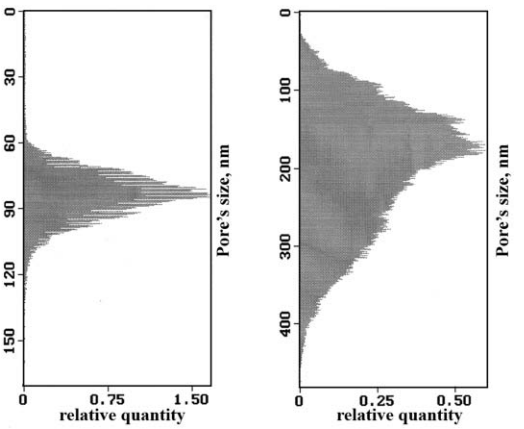

Fig. 4 Size distribution of sub-micropores in cartilage matrix before (on the left) and after (on the right) laser irradiation of AF of a rabbit intervertebral disk.

Thus, one of the possible contributions of laser radiation to regeneration processes is acceleration of mass transfer through ECM. Accelerated mass transfer in various solids under laser radiation was long studied (see Ref. 60 and referred literature). The highest observed values of the effective transfer coefficient ($\sim$0.1 cm^2/s) are usually associated with the melting and hydrodynamic process of melt flow. However, abnormally high mass transfer coefficients were also observed to occur in the course of laser processes in multicomponent solids involving no liquid phase formation. This phenomenon was detected only for heterogeneous materials. It was hypothesized on the basis of great impact of the pore system resulted from thermo mechanical stress under nonuniform laser heating.[60]

Both existing pores and the pores due to nonuniform laser heating, such as, short-lived channels, closing soon after laser irradiation, may play a role. In biopolymers, like cartilage matrix, the temporal pores could be large enough to provide motion of relatively large objects, such as cartilaginous cells to the areas of low cellularity. This process may be considered as one of the possible reasons for the emergence of chondrocytes in the nucleus pulposus of the intervertebral disk after laser irradiation[61,62] (see Sec. 5.2). There is an optimal range of the pore size. Wide pores in ECM, in general, are nondesirable because they decrease cartilage durability and allow transfer of large molecules into the bulk of cartilage, potentially resulting in the formation of abnormal structures containing blood vessels and/or nerves. The nucleation and development of the pores in cartilage interplay among gas bubbles existing in the tissue or arising in the course of laser irradiation due to water boiling or (at lower temperatures) due to the temperature dependency of gas solubility in the tissue. The microscopic gas bubbles in interstitial liquid can be stabilized by positive ions bound with the bubble surface.[63] The growth and movement of gas bubbles in cartilage under nondestructive laser radiation were demonstrated in Refs. 56 and 57. Since cellular metabolism and activity depends on oxygen concentration in cartilage,[64] the gas bubbles formation and movement under nonablative laser radiation may have a controlling effect on the oxygen concentration and, therefore, on the processes of cellular differentiation/dedifferentiation, proliferation, and new matrix production.

Another mechanism of laser-induced activation of the regeneration processes in cartilage can be resulted from the

mechanical effect on chondrocytes due to gas bubbles oscillation and movement under nonhomogeneous pulse periodic laser heating.[57] It is known that chondrocytes are very sensitive to external mechanical forces. In particular, oscillating mechanical pressure of specific frequencies and amplitudes can stimulate chondrocyte proliferation and the production of collagen and PGs.[31,65,66] The pulse repetition frequencies used for cartilage regeneration in animal spine disks and joints are in the range from 0.2 to 1.2 Hz, which corresponds with the experimental results obtained for chondrocyte cultures.[67] The motility of tissue water and gas bubbles under nonuniform laser heating may also have a positive effect on the cells. Computational modeling[68] demonstrates that when morphogens are autologously secreted by a cell in matrix-binding form and under low levels of interstitial flow, morphogens' gradients develop to guide cell processes in the direction of the flow. Thus, temporally and spatially modulated laser radiation can provide precise control of different parameters, which is important for the regeneration process, i.e., temperature, amplitude and frequency of the mechanical effect, formation of morphogenetic gradients, and mass transfer to and from the cells.

5 Experimental and Clinical Studies. Prospects of Laser Regeneration of Cartilage

5.1 *Laser-induced Regeneration of Articular Cartilage*

The widespread and obstinate disease of joints, osteoarthritis (OA),[69,70] may be caused by trauma, infection, metabolic and endocrine dysfunctions, and other reasons. The most common pathological feature of the post-traumatic OA is a lesion of the articular cartilage plate. If these lesions are relatively large (more than 3 mm in size) and superficial (partial-thickness defects that do not reach the bone), they never repair without external intervention. More deep, full-thickness injuries are usually covered with fibrous tissue or trabecular bone. In spite of many efforts and intensive research, the problem of the treatment of small or extensive defects of the cartilage is not solved yet. Literature reviews of laser applications for tissue repair, including articular cartilage, are presented in Refs. 71 and 72. It was concluded that many studies are not scientifically substantiated and future investigations should be based on sound biological foundations. Later, Athanasiou et al.[73] studied the effect of an excimer laser on the healing of articular cartilage in a rabbit's knee. Chondral shaving and subchondral abrasion of cartilage, by creating partial-thickness and full-thickness cartilage defects of standardized size, were imitated with both an excimer laser and drilling. Examinations of the repair tissue showed that healing of osteochondral defects created by a laser may be delayed compared with injuries inflicted by drilling, postoperatively for at least six weeks. Even though there initially was a considerable delay in healing in the laser group, neither laser nor drilling had any appreciable effects on the mechanical properties of the repair tissue, as demonstrated by biomechanical testing at 14 weeks.

The long duration set of *in vivo* studies aimed to evaluate the nonablative laser effect on experimentally traumatic osteoarthritis of knee joints has been carried out in minipigs.[74,75] The excisional defects that had a size of 3 to 15 mm and depth of 0.5 to 0.6 mm (partial-thickness lesions) or up to 1 mm (full-thickness defects) were created on the head of the femoral bone. The typical appearance of experimental injuries is shown in Fig. 5. Six weeks later, the joints were re-opened and visually examined. The original defects were still clearly discernible. Moreover, in some operated joints, the secondary lesions appeared in the zones of maximal physiological loading adjacent to the primary injuries. The depth of the secondary lesions was about 0.2 to 0.4 mm. The laser treatment of the initial and secondary defects was performed using the Erbium-glass fiber laser (Arcuo Medical Inc.) with a wavelength of 1.56 microns, pulse duration of 100 ms, pulse repetition rate of 0.7 Hz, and laser beam diameter of 600 microns.[74,75] The temperature during the laser treatment detected by thin thermocouple and IR radiometer, reached 50 °C. Spatial modulation of laser irradiation provided temperature gradients of an order of 100 degrees/cm, while temporal modulation produced the thermo mechanical effects at a frequency of 0.7 Hz. The animals were sacrificed after two, three, and six months following the laser treatment or sham surgery (in the control group); and the biopsies of the laser-irradiated areas of articular cartilage were histologically examined versus control defects.

It was revealed that in contrast to untreated lesions, the majority of laser-irradiated primary and secondary defects were filled (wholly or in a part) with hyaline-like cartilage (Fig. 5). This new tissue possessed typical homogeneous ECM and numerous chondrocytes predominantly located in lacunae (Fig. 6). The intensified biosynthesis of the PGs was also detected. The cellular proliferation and matrix accumulation resulted in the increase of new cartilage volume up to the values of 80 to 100% of the initial cubature of the defects. The thickness of neogenetic cartilage decreases from the periphery to the center of the lesion. This can be evidenced in favor of the hypothesis that regeneration starts not from the bottom of the damaged area, but mainly from the margins of the lesion and spreads toward its center implying activation of a reparative response even outside the laser-irradiated area. In a control group, the partial-thickness defects (both primary and secondary) retained their initial size in the absence of new tissue growth up to 30 weeks after the first operation. The mechanical damage of the defects' bottoms, dystrophic, and necrotic alterations of cartilage near the lesions, including decrease in the PGs content, were observed. The bottom of some full-thickness primary injuries was covered with fibrous connective tissue (up to 20% of the depth). It is worth emphasizing that the restoration of LS was observed in the majority of laser-treated defects. Although LS is of great importance to provide lubrication and mechanical properties of articular cartilage, it is

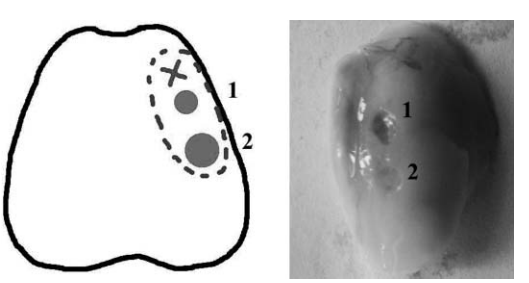

Fig. 5 Repair of the defect of porcine articular cartilage in two months after laser treatment. 1- control (nonirradiated defect of 4 mm in diameter), 2- the laser-treated defect of 6 mm in diameter is filled with a new growing tissue.

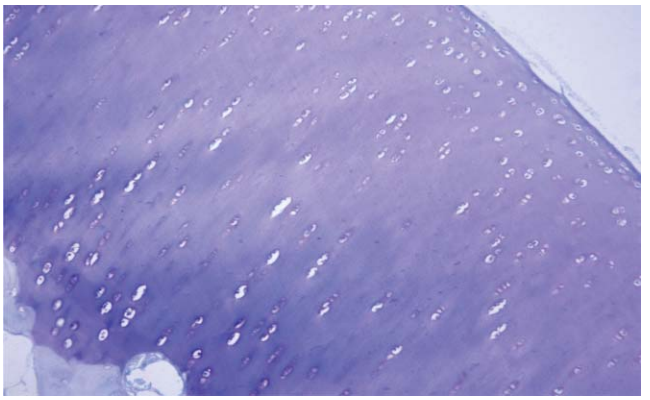

Fig. 6 Hyaline cartilage replacing partial-thickness defect of porcine articular cartilage in three months after laser irradiation. The restoration of the lamina splendence at the cartilage surface is seen. Toluidine blue staining, ×200.

never restored after the replacement of the damaged areas with fibrous cartilage or fibrous connective tissue. The restore of the LS is important evidence of advanced character of articular cartilage regeneration under laser radiation. Thus, *in vivo* animal experiments on laser-induced regeneration of hyaline articular cartilage have demonstrated[74,75] promising results for primary (traumatic) as well as for secondary (degenerative) injuries.

5.2 *Laser Reconstruction of Spine Disks*

Recently, lasers have been used for treatment of spine disk problems.[76,77] Removal of herniations, decompression, and denervation of the disk are the major approaches in this field. These techniques are based, in general, on the significant heating and denaturation of the anulus fibrosus (AF) tissue, inevitably diminishing mechanical strengh of the AF, which may result in various late complications. So, none of the above laser techniques enables one to obtain a satisfactory solution to the problem of back pain and disk-related disability, considering the fact that in some cases the intervertebral disk hernia relapses and recurrent pains come back in a year after laser surgery.

Another approach to the treatment of spinal diseases based on the thermo mechanical effect of nondestructive laser radiation on nucleus pulposus (NP) of the intervertebral disk was introduced in 2000.[62] *In vivo* animal studies showed that depending on laser settings, nonablative laser treatment triggers growth of fibrous or hyaline cartilages.[61,62] Experiments in rabbits demonstrated the growth of neogenetic cartilage after nonablative laser treatment of the spinal disks in only the irradiated zones.[55,73,78,79] The histological and electron microscopic examinations verified formation of hyaline-like and fibrous-hyaline cartilages (Figs. 7 and 8) in the modeled defects of the NP in two and six months after laser treatment. The chondrocyte-like cells or resident stem cells of the NP, as well as immature chondrocytes migrating from the endplates, undergoing the differentiation as a result of thermo mechanical effect of the laser radiation may represent sources of new hyaline-like cartilage growth. New chondrocytes can be distinguished from the cells of untreated zones by their active synthesis of PGs. An important result of laser irradiation is also the recovery of the hyaline endplates, which were damaged following the model degenerative process

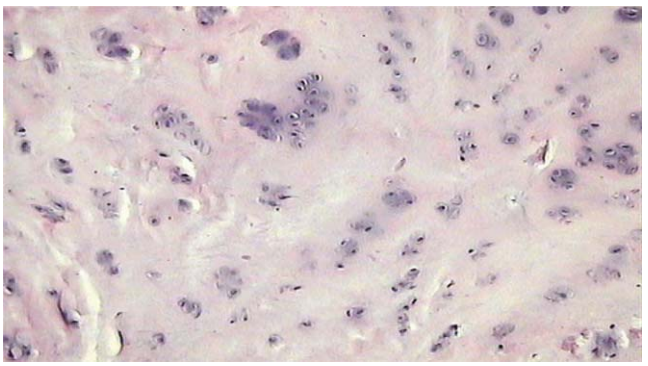

Fig. 7 Hyaline-like cartilage tissue growing in the laser irradiated area of a rabbit intervertebral disk in a month after laser irradiation. Hematoxylin and eosin staining, ×200.

and bore the pronounced signs of destruction and calcinosis before the treatment.

The results of the above-mentioned experiments allowed the establishment of the laser settings (power, exposure time and parameters of temporal modulation of the laser beam) for hyaline cartilage regeneration without significant damage of the AF and gave grounds for a new minimally invasive technology of laser reconstruction of the disks (LRD).[55,73,78,79] In the course of LRD, laser radiation of 1.5 W in power was delivered to the NP of the damaged disk through an optical fiber using a needle puncture. All zones of the NP were irradiated by a series of laser pulses (pulse duration was of 2 s, interval between pulses was of 1 s). The resulting frequency of thermo mechanical pulses was 0.33 Hz, close to the optimal frequency interval found for chondrocytes.[67] The equipment for LRD (Arcuo Medical Inc.) includes a 1.56 microns laser, a disposable fiber instrument, and a feedback control system based on the monitoring of the backscattered light signal during laser treatment.[74] Following clinical data, 540 patients have received LRD in 2006 to 2010. Pain relief and return to normal life with five years follow-up have been

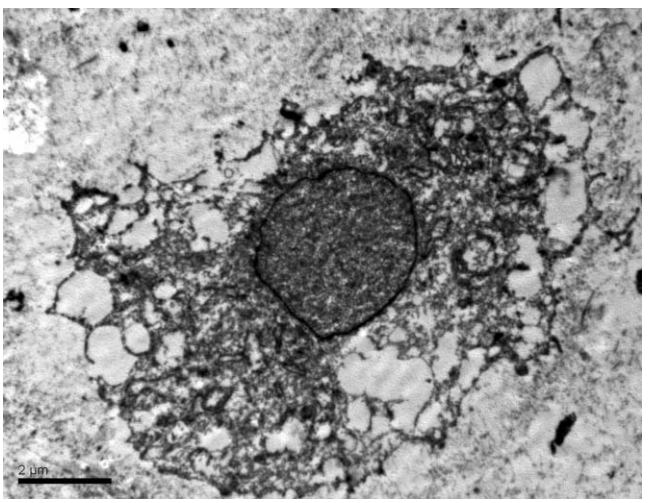

Fig. 8 Chondrocyte of regenerated hyaline-like cartilage in a rabbit intervertebral disk in three months after laser irradiation. Well-developed rough endoplasmic reticulum is visible inside the cell surrounded by pericellular matrix and capsule. TEM image. Scale bar 2 μm.

demonstrated in more than 90% of the treated patients. Long-term stability of the positive outcomes of LRD and the growth of hyaline-like cartilage in laser treated zones of spine disks was confirmed in human studies as was previously established in the animal models.[55,56,79] The above characteristics of LRD are advantageous compared to other minimal invasive techniques of spine diseases treatment, which mainly provide heating of the AF and denervation of the disks. Laser wavelength and other characteristics for LRD have been chosen to locally heat NP in the bulk of about 1 mm^3 up to temperatures of about 50 °C for a short period of time when the damage and denaturation of the disk tissue do not have enough time to develop.[55–57] The expansion of locally heated tissue provides thermo mechanical stress at a distance of about a few millimeters from the zone of laser irradiation. LRD results in the formation of well-organized hyaline-like tissue, probably because the effect of laser radiation in the course of LRD is mild enough. We suggest that laser irradiation (in terms of LRD) leads to differentiation of resident chondrocyte-like and stem cells of NP as well as to the migration of immature cartilage cells from hyaline endplates due to ECM modification and the formation of the morphogenetic gradients. In addition, LRD provides nutrition for the chondrocytes due to pore formation and also probably activates the cells of NP toward activation of biosynthesis of ECM components (Fig. 3) by the means of mechanical oscillations and mass-transfer effects. The laser technique determines necessary prerequisites for application of the optical-based feedback control systems, which allow monitoring of important parameters of the treated tissue (temperature, light scattering, and electrical impedance). Measurements of optical characteristics in the course of laser treatment give information on the modification of cartilage matrix structure and produce signals for laser parameters adjustment and for switching the laser off.[57,74]

5.3 *Laser Regeneration of Cartilage in Otolaryngology*

Laser reshaping of cartilage is a new bloodless and painless technology for controlled correction of cartilage shape as a result of nondestructive modification of tissue structure and stress distribution.[60,80,81] It was first used in clinics for the correction of nasal septum deformities and for reshaping ears.[82–85] The prospects of laser reshaping of costal and tracheal cartilages as well as the epiglottis have also been demonstrated.[85–87]

The histological results of *in vivo* experiments on laser reshaping of porcine ears have shown the growth of hyaline cartilage at the periphery of the zone where laser-induced transformation of cartilage matrix structure occurred.[50] Since the cells can undergo some effect of laser radiation in the course of the laser reshaping of cartilage, the regeneration processes may alter the tissue shape immediately obtained after the laser correction procedure. Hence, the cartilage regeneration process may determine the stability of the final shape and, therefore, the time period of the post-operation maintenance of the new cartilage configuration. Such time can vary from a few hours up to two weeks for different laser settings.[84,88] Thus, the precise choice of laser parameters allows control of the extent of the neogenetic tissue emerged as a result of laser-induced regeneration process.

Laser-induced regeneration of cartilage may also play an important role in the solution of the problem of surgical treatment of the tracheal stenosis.[89,90] The degree of the tracheal stenosis depends on the extent of tracheal injury as it was shown for the laser cauterization of the tracheal rings. One of the current approaches of tracheal stenosis treatment is costal cartilage implantation to fill the defect in the larynx.[91] The main obstacle for this method is growth of fibrous tissue that can result in stenosis relapse requiring additional surgeries. Optimization of laser settings for cartilage reshaping can be important to provide better conditions for cartilage engraftment allowing one to avoid staged surgical procedures.

6 Summary

Regeneration is a natural process, an essential respond of the living tissue on almost any external physical or chemical effect turning the system out the equilibrium and leading to the repair of the destroyed tissues. Laser radiation is a convenient and efficient tool that allows control of some essential processes of tissue regeneration. Since the strict natural limits of cartilage regeneration do exist, the laser radiation may control some latent resources of cellular supply and metabolism of the cartilage structures. Three main interrelated processes contribute to the effect of nonablative laser radiation on cartilage tissue. These are nonuniform heating, mass-transfer, and control of mechanical stress in the tissue. Nonablative character of the radiation allows physical and chemical modifications of ECM, including development of a fluctuating porous system as well as controllable replacements of interstitial water, contributing to morphogenetic gradient formation and to the developmental role of mechanical pressure and tensional loads.

The main advantage of laser-induced regeneration is triggering of the reactions, which lead to filling of the defects of cartilage with hyaline-like tissue. The basic biological mechanisms of these reactions are stimulation of differentiation of resident immature and stem cells and increase of accumulation of ECM components by the hyaline cartilage chondrocytes. We also cannot exclude possible laser induction of limited dedifferentiation of the mature chondrocytes to rejuvenation of the cellular population of the tissue toward recovery of their ability to divide and restore the lost tissue volume. According to the state-of-the-art conceptions, the absolute majority of those reactions may be mediated through the changes that occur in ECM of cartilage under spatial and temporal modulated laser radiation.

It is necessary to note that some conceptions discussed above have their bases in incomplete data and require future detailed studies. In spite of the fact that a lot of unstudied processes still exist, the prospects of laser-induced regeneration of cartilage are undoubted. All the hyaline cartilage structures have similar morphological organization and vital functions. The differences mainly relate to the cellular population features and to the spatial organization of the tissues. Nevertheless, the ECM of hyaline cartilage of the ribs, joints, and nasal septae is quite similar. Therefore, the mechanisms of laser-induced regeneration considered above can be extended to the cartilages of different localizations.

The potential safety of laser regeneration is connected with the nondestructive character of laser effect on tissue matrix and with arrangement of favorable conditions for cell proliferation and functioning. As laser-induced regeneration of cartilage

requires precise control of the optimal laser settings, the development of feedback control systems, in particular, based on optical measurements, is important to guarantee efficacy and safety of the laser application in clinics.

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