Cartilage Regeneration – Shaping – Collagen Effects

Photomed Laser Surg. 2010 Aug;28(4):527-32.

Effect of GaAlAs Laser Irradiation on the Epiphyseal Cartilage of Rats.

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Abstract

Abstract Objective: To study the effect of an 830-nm gallium-aluminum-arsenic (GaAlAs) diode laser at two different energy densities (5 and 15 J/cm(2)) on the epiphyseal cartilage of rats by evaluating bone length and the number of chondrocytes and thickness of each zone of the epiphyseal cartilage. Background Data: Few studies have been conducted on the effects of low-level laser therapy on the epiphyseal cartilage at different irradiation doses. Materials and Methods: A total of 30 male Wistar rats with 23 days of age and weighing 90 g on average were randomly divided into 3 groups: control group (CG, no stimulation), G5 group (energy density, 5 J/cm(2)), and G15 group (energy density, 15 J/cm(2)). Laser treatment sessions were administered every other day for a total of 10 sessions. The animals were killed 24 h after the last treatment session. Histological slides of the epiphyseal cartilage were stained with hematoxylin-eosin (HE), photographed with a Zeiss photomicroscope, and subjected to histometric and histological analyses. Statistical analysis was performed using one-way analysis of variance followed by Tukey's post hoc test. All statistical tests were performed at a significance level of 0.05. Results: Histological analysis and x-ray radiographs revealed an increase in thickness of the epiphyseal cartilage and in the number of chondrocytes in the G5 and G15 groups. Conclusion: The 830-nm GaAlAs diode laser, within the parameters used in this study, induced changes in the thickness of the epiphyseal cartilage and increased the number of chondrocytes, but this was not sufficient to induce changes in bone length.

J Photochem Photobiol B. 2010 Mar 8;98(3):211-5. Epub 2010 Jan 25.

Chondrogenic mRNA expression in prechondrogenic cells after blue laser irradiation.

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Abstract

Low-level laser therapy (LLLT) has been used as a method for biostimulation. Cartilage develops through the differentiation of mesenchymal cells into chondrocytes, and differentiated chondrocytes in articular cartilage maintain cartilage homeostasis by synthesizing cartilage-specific extracellular matrix. The aim of this study is to evaluate the enhancement of chondrocyte differentiation and the expression levels of chondrogenic mRNA in prechondrogenic ATDC5 cells after laser irradiation. For chondrogenic induction, ATDC5 cells were irradiated with a blue laser (405 nm, continuous wave) at 100 mW/cm(2) for 180 s following incubation in chondrogenic differentiation medium. Differentiation after laser irradiation was quantitatively evaluated by the measurement of total collagen contents and chondrogenesis-related mRNAs. The total amount of collagen and mRNA levels of aggrecan, collagen type II, SOX-9, and DEC-1 were increased relative to those of a non-laser irradiated group after 14 days of laser irradiation. On the other hand, Ap-2alpha mRNA, a negative transcription factor of chondrogenesis, was dramatically decreased after laser irradiation. In addition, intracellular reactive oxygen species (ROS) were generated after laser irradiation. These results, for the first time, provide functional evidence that mRNA expression relating to chondrogenesis is increased, and Ap-2alpha is decreased immediately after laser irradiation. As this technique could readily be applied in situ to control the differentiation of cells at an implanted site within the body, this approach may have therapeutic potential for the restoration of damaged or diseased tissue.

Lasers Surg Med. 2009 Sep;41(7):487-91.

The effects of laser irradiation of cartilage on chondrocyte gene expression and the collagen matrix.

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Abstract

OBJECTIVES: Laser reshaping of cartilage is an emerging technology aimed at replacing conventional techniques for aesthetic and reconstructive surgery. Little is known about the mechanisms of wound healing following the photothermal heating during laser reshaping and, ultimately, how collagen remodels in the irradiated tissue. Healthy hyaline and elastic cartilage as found in the ear, nose, larynx, and trachea does not express collagen type I which is characteristic of fibro-cartilage and scar tissue. The aim of the study was to determine if collagen I and II gene expression occurs within laser irradiated rabbit septal cartilage.

METHODS: Nasal septum harvested from freshly euthanized New Zealand White rabbits were irradiated with an Nd:YAG laser. After 2 weeks in culture, the laser spot and surrounding non-irradiated regions were imaged using immunofluorescence staining and evaluated using reverse transcription polymerase chain reaction (RT-PCR) to determine the presence of collagen I and II, and ascertain collagen I and II gene expression, respectively.

RESULTS: All laser irradiated specimens showed a cessation in collagen II gene expression within the center of the laser spot. Collagen II was expressed in the surrounding region encircling the laser spot and within the non-irradiated periphery in all specimens. Immunohistochemistry identified only type II collagen. Neither collagen I gene expression nor immunoreactivity were identified in any specimens regardless or irradiation parameters.

CONCLUSIONS: Laser irradiation of rabbit septal cartilage using dosimetry parameters similar to those used in laser reshaping does not result in the detection of either collagen I gene expression or immunoreactivity. Only collagen type II was noted after laser exposure in vitro following cell culture, which suggests that the cellular response to laser irradiation is distinct from that observed in conventional wound healing. Laser irradiation of cartilage can leave an intact collagen matrix which likely allows chondrocyte recovery on an intact scaffold.

J Photochem Photobiol B. 2007 Jul 27;88(1):11-5. Epub 2007 May 1.

The therapeutic effect of low-level laser on repair of osteochondral defects in rabbit knee.

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INTRODUCTION: Low level laser therapy (LLLT) has been shown to enhance collagen production and wound healing but its effect on cartilage repair from biomechanical point of view is not known yet. The aim of present study was to evaluate the biomechanical behaviour of repairing osteochondral defect in rabbits which received a pulsed low-level gallium-arsenide (Ga-As) laser irradiation. MATERIALS AND METHODS: Osteochondral defects with 5mm diameter and 4mm in depth induced by drilling in right femoral patellar grooves of 41 adolescent male rabbits. They were divided into experimental and control groups. Experimental group received pulsed Ga-As (890nm) laser irradiation with energy density of 4.8J/cm(2). The rabbits in control group received placebo LLLT with shut-down equipment. The control defects were allowed to heal spontaneously. Each group were divided into three subgroups: A, B and C. Subgroups A, B and C were sacrificed on 4, 8, and 16 weeks after surgery. The knee joint were removed, and the defects were examined biomechanically by in situ-indentation method. The thickness, instantaneous and equilibrium indentation stiffness was measured during the test. Data were analysed using ANOVA and independent sample t-test. RESULT: While no difference was observed in the repaired cartilage biomechanical properties among 4th, 8th, 16th weeks in study groups. The equilibrium indentation stiffness of experimental group was significantly higher in 8th week in comparison with control group. CONCLUSION: LLLT significantly enhances the stiffness of repairing tissue in the 8th week post injury in osteochondral defects in rabbits.

Photomed Laser Surg. 2006 Dec;24(6):754-8

Comparative Study Using 685-nm and 830-nm Lasers in the Tissue Repair of Tenotomized Tendons in the Mouse.

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Objective: The objective of this study was to evaluate the effects of 685- and 830-nm laser irradiations, at different fluences on the healing process of Achilles tendon (Tendon calcaneo) of mice after tenotomy.

Background Data: Some authors have shown that low-level laser therapy (LLLT) is able to accelerate the healing process of tendinuos tissue after an injury, increasing fibroblast cell proliferation and collagen synthesis. However, the mechanism by which LLLT acts on healing process is not fully understood. Methods: Forty-eight male mice were divided into six experimental groups: group A, tenomized animals, treated with 685 nm laser, at the dosage of 3 J/cm(2); group B, tenomized animals, treated with 685-nm laser, at the dosage of 10 J/cm(2); group C, tenomized animals, treated with 830-nm laser, at dosage of 3 J/cm(2); group D, tenomized animals, treated with 830-nm laser, at the dosage of 10 J/cm(2); group E, injured control (placebo treatment); and group F, non-injured standard control. Animals were killed on day 13 post-tenotomy, and their tendons were surgically removed for a quantitative analysis using polarization microscopy, with the purpose of measuring collagen fibers organization through the birefringence (optical retardation [OR]).

Results: All treated groups showed higher values of OR when compared to injured control group. The best organization and aggregation of the collagen bundles were shown by the animals of group A (685 nm, 3 J/cm(2)), followed by the animals of group C and B, and finally, the animals of group D.

Conclusion: All wavelengths and fluences used in this study were efficient at accelerating the healing process of Achilles tendon post-tenotomy, particularly after the 685-nm laser irradiation, at 3 J/cm(2). It suggests the existence of wavelength tissue specificity and dose dependency. Further studies are required to investigate the physiological mechanisms responsible for the effects of laser on tendinuos repair.

Lasers Surg Med. 2005 Jul;37(1):89-96.

Identification of chondrocyte proliferation following laser irradiation, thermal injury, and mechanical trauma.

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Abstract

BACKGROUND AND OBJECTIVE: Cartilage has a limited regenerative capacity, and there are a lack of reliable techniques and methods to stimulate growth of new tissue to treat degenerative diseases and trauma. This study focused on identifying chondrocyte cell proliferation in ex vivo cartilage tissue following heating Nd:YAG laser using whole-mount analysis and flow cytometry, and compared findings with results produced by contact, and water bath heating methods, mechanical injury, and the addition of transforming growth factor-beta (TGF-beta).

STUDY DESIGN/MATERIALS AND METHODS: Ex vivo rabbit nasal septal cartilages were either irradiated with an Nd:YAG laser (lambda = 1.32 microm, 2-16 seconds, 6 W/cm(2)), heated by immersion in a warm saline bath, heated by direct contact with a metal rod, or mechanically damaged by scoring with a scalpel or crushing. After treatment, specimens were incubated for 7 or 14 days in growth media containing 10 microM bromodeoxyuridine (BrdU). Additional specimens were cultured with both BrdU and TGF-beta. Both whole-mount BrdU-double-antibody detection techniques and flow cytometry were used to determine the presence of DNA replication as a marker of proliferation.

RESULT: An annular region of regenerating chondrocytes was identified surrounding the laser irradiation zone in whole-mount tissue specimens, and the diameter of this region increased with irradiation time. Using whole-mount analysis, no evidence of chondrocyte DNA replication was observed in tissues heated using non-laser methods, grown in TGF-beta, or mechanically traumatized. In contrast, flow cytometry identified the presence of BrdU-positive cells in the S-phase of the cell cycle (synthesis of DNA) for all protocols, indicating chondrocyte proliferation. The percentage of cells that are in S-phase increased with irradiation time.

CONCLUSION: These data provide evidence that laser irradiation, along with other thermal and mechanical treatments, causes a proliferative response in chondrocytes, and this is observed ex vivo in the absence of cellular and humoral repair mechanisms. The advantage of using optical methods to generate heat in cartilage is that microspot injuries could be created in tissue and scanned across surfaces in clinical applications.

Lasers Surg Med. 2008 Mar;40(3):202-10.

Temperature dependent change in equilibrium elastic modulus after thermally induced stress relaxation in porcine septal cartilage.

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Abstract

BACKGROUND AND OBJECTIVES: Laser cartilage reshaping (LCR) is a promising method for the in situ treatment of structural deformities in the nasal septum, external ear and trachea. Laser heating leads to changes in cartilage mechanical properties and produces relaxation of internal stress allowing formation of a new stable shape. While some animal and preliminary human studies have demonstrated clinical feasibility of LCR, application of the method outside specialized centers requires a better understanding of the evolution of cartilage mechanical properties with temperature. The purpose of this study was to (1) develop a method for reliable evaluation of mechanical changes in the porcine septal cartilage undergoing stress relaxation during laser heating and (2) model the mechanical changes in cartilage at steady state following laser heating.

STUDY DESIGN/MATERIALS AND METHODS: Rectangular cartilage specimens harvested from porcine septum were heated uniformly by a radio-frequency (RF) electric field (500 kHz) for 8 and 12 seconds to maximum temperatures from 50 to 90 degrees C. Cylindrical samples were fashioned from the heated specimens and their equilibrium elastic modulus was measured in a step unconfined compression experiment. Functional dependencies of the elastic modulus and maximum temperature were interpolated from the measurements. Profiles of the elastic modulus produced after 8 and 12 seconds of laser irradiation (Nd:YAG, lambda = 1.34 microm, spot diameter 4.8 mm, laser power 8 W) were calculated from interpolation functions and surface temperature histories measured with a thermal camera. The calculated elastic modulus profiles were incorporated into a numerical model of uniaxial unconfined compression of laser irradiated cylindrical samples. The reaction force to a 0.1 compressive strain was calculated and compared with the reaction force obtained in analogous mechanical measurements experiment.

RESULTS: RF heating of rectangular cartilage sample produces a spatially uniform temperature field (temperature variations < or = 4 degrees C) in a central region of the sample which is also large enough for reliable mechanical testing. Output power adjustment of the RF generator allows production of temperature histories that are very similar to those produced by laser heating at temperatures above 60 degrees C. This allows creation of RF cartilage samples with mechanical properties similar to laser irradiated cartilage, however with a spatially uniform temperature field. Cartilage equilibrium elastic modulus as a function of peak temperature were obtained from the mechanical testing of RF heated samples. In the temperature interval from 60 to 80 degrees C, the equilibrium modulus decreased from 0.08+/- 0.01 MPa to 0.016+/-0.007 MPa, respectively. The results of the numerical simulation

of uniaxial compression of laser heated samples demonstrate good correlation with experimentally obtained reaction force.

CONCLUSIONS: The thermal history and corresponding thermally induced modification of mechanical properties of laser irradiated septal cartilage can be mimicked by heating tissue samples with RF electric current with the added advantage of a uniform temperature profile. The spatial distribution of the mechanical properties obtained in septal cartilage after laser irradiation could be computed from mechanical testing of RF heated samples and used for numerical simulation of LCR procedure. Generalization of this methodology to incorporate orthogonal mechanical properties may aid in optimizing clinical laser cartilage reshaping procedures.

Lasers Surg Med. 2005 Oct;37(4):293-300

Low-level laser therapy (LLLT) prevents oxidative stress and reduces fibrosis in rat traumatized Achilles tendon.

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BACKGROUND AND OBJECTIVES: The present study investigated the effects of low-level laser therapy (LLLT) on oxidative stress and fibrosis in an experimental model of Achilles tendon injury induced by a single impact trauma. STUDY DESIGN/MATERIALS AND METHODS: Male Wistar rats were randomly divided into four groups (n = 8): control, trauma, trauma+LLLT for 14 days, and trauma+LLLT for 21 days. Achilles tendon traumatism was produced by dropping down a load with an impact kinetic energy of 0.544 J. A low level Ga-As laser was applied with a 904 nm wavelength, 45 mW average power, 5 J/cm(2) dosage, for 35 seconds duration, continuously. Studies were carried out at day 21. RESULTS: Histology showed a loss of normal architecture, with inflammatory reaction, angiogenesis, vasodilatation, and extracellular matrix formation after trauma. This was accompanied by a significant increase in collagen concentration when compared the control group. Oxidative stress, measured by the concentration of thiobarbituric acid reactive substances and hydroperoxyde-initiated chemiluminiscence, was also significantly increased in the trauma group. Administration of LLLT for 14 or 21 days markedly alleviated histological abnormalities reduced collagen concentration and prevented oxidative stress. Superoxide dismutase activity was significantly increased by LLLT treatment over control values. CONCLUSION: LLLT by Ga-As laser reduces histological abnormalities, collagen concentration, and oxidative stress in an experimental model of Achilles tendon injury. Reduction of fibrosis could be mediated by the beneficial effects on the oxidant/antioxidant balance.

Photomed Laser Surg. 2004 Aug;22(4):323-9.

The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study.

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Abstract

OBJECTIVE: We used statistical meta-analysis to determine the overall treatment effects of laser phototherapy on tissue repair and pain relief.

BACKGROUND DATA: Low-power laser devices were first used as a form of therapy more than 30 years ago. However, their efficacy in reducing pain or promoting tissue repair remains questionable.

METHODS: Following a literature search, studies meeting our inclusion criteria were identified and coded. Then, the effect size of laser treatment, that is, Cohen's d, was calculated from each study using standard meta-analysis procedures.

RESULTS: Thirty-four peer-reviewed papers on tissue repair met our inclusion criteria and were used to calculate 46 treatment effect sizes. Nine peer-reviewed papers on pain control met the inclusion criteria and were used to calculate nine effect sizes. Meta-analysis revealed a positive effect of laser phototherapy on tissue repair (d = +1.81; n = 46) and pain control (d = +1.11; n = 9). The positive effect of treatment on specific indices of tissue repair was evident in the treatment effect sizes determined as follows: collagen formation (d = +2.78), rate of healing (d = +1.57), tensile strength (d = +2.13), time needed for wound closure (d = +0.76), tensile stress (d = +2.65), number and rate of degranulation of mast cells (d = +1.87), and flap survival (d = +1.95). Further, analysis revealed the positive effects of various wavelengths of laser light on tissue repair, with 632.8 nm having the highest treatment effect (d = +2.44) and 780 nm the least (d = 0.60). The overall treatment effect for pain control was positive as well (d = +1.11). The fail-safe number-that is, the number of studies in which laser phototherapy has negative or no effect-needed to nullify the overall outcome of this analysis was 370 for tissue repair and 41 for pain control.

CONCLUSIONS: These findings mandate the conclusion that laser phototherapy is a highly effective therapeutic armamentarium for tissue repair and pain relief.

Lasers Surg Med. 2004;34(4):323-8.

Effect of low-power He-Ne laser irradiation on rabbit articular chondrocytes in vitro.

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Retraction in:

- Lasers Surg Med. 2005 Oct;37(4):330.

Abstract

BACKGROUND AND OBJECTIVES: In the orthopaedic field, the repair of articular cartilage is still a difficult problem, because of the physiological characters of cartilaginous tissues and chondrocytes. To find an effective method of stimulating their regeneration, this in vitro study focuses on the biostimulation of rabbit articular chondrocytes by low-power He-Ne laser.

STUDY DESIGN/MATERIALS AND METHODS: The articular chondrocytes isolated from the cartilage of the medial condyle of the femur of the rabbit were incubated in DMEM/HamF(12) medium. The second passage culture were spread on 24 petri dishes and were irradiated with laser at power output of 2-12 mW for 6.5 minutes, corresponding to the energy density of 1-6 J/cm(2). Laser treatment was performed three times at a 24-hour interval. After lasering, incubation was continued for 24 hours. Non-irradiated cells were kept under the same conditions as the irradiated ones. The cell proliferation activity was evaluated with a XTT colorimetric method and the cell secretion activity was analyzed by metachromasia and immunocytochemistry.

RESULTS: Irradiation of 4-6 J/cm(2) increased the cell numbers and revealed a considerably higher cell proliferation activity comparing to control cultures. Thereinto, the energy density of 4 and 5 J/cm(2) remarkably increased cell growth, with positive effect on synthesis and secretion of extracellular matrix.

CONCLUSIONS: The present study showed that a particular laser irradiation stimulates articular chondrocytes proliferation and secretion. These findings might be clinically relevant, indicating that low-power laser irradiation treatment is likely to achieve the repair of articular cartilage in clinic.

Indian J Exp Biol. 2004 Sep;42(9):866-70.

Effect of low-power helium-neon laser irradiation on 13-week immobilized articular cartilage of rabbits.

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Abstract

Influence of low-power (632.8 nm, Helium-Neon, 13 J/cm2, three times a week) laser on 13-week immobilized articular cartilage was examined with rabbits knee model. Number of chondrocytes and depth of articular cartilage of experimental group were significantly higher than those of sham irradiated group. Surface morphology of sham-irradiated group had rough prominences, fibrillation and lacunae but surface morphology of experimental group had more similarities to control group than to sham irradiated group. There were marked differences between ultrastructure features of control group and experimental group in comparison with sham irradiated group. Low-power Helium-Neon laser irradiation on 13-week immobilized knee joints of rabbits neutrilized adverse effects of immobilization on articular cartilage.

Lasers Surg Med. 2003;32(1):3-9.

Quantitative assessment of chondrocyte viability after laser mediated reshaping: a novel application of flow cytometry.

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Abstract

BACKGROUND AND OBJECTIVES: Lasers can be used to reshape cartilage by accelerating mechanical stress relaxation. In this study, fluorescent differential cell viability staining and flow cytometry were used to determine chondrocyte viability following laser heating.

STUDY DESIGN/MATERIALS AND METHODS: Porcine septal cartilages were irradiated with an Nd:YAG laser (lambda = 1.32 microm, 25 W/cm(2)) while surface temperature, stress relaxation, and diffuse reflectance were recorded. Each slab received one, two, or three laser exposures (respective exposure times of 6.7, 7.2, 10 seconds). Irradiated samples were then divided into two groups analyzed immediately and at 5 days following laser exposure. Chondrocytes were isolated following serial enzymatic digestion, and stained using SYTO/DEAD Red (Molecular Probes, Eugene, OR). A flow cytometer was then used to detect differential cell fluorescence; size; granularity; and the number of live cells, dead cells, and post-irradiation debris in each treatment population.

RESULTS: Nearly 60% of chondrocytes from reshaped cartilage samples isolated shortly after one irradiation, were viable while non-irradiated controls were 100% viable. Specimens irradiated two or three times demonstrated increasing amounts of cellular debris along with a reduction in chondrocyte viability: 31 and 16% after two and three exposures, respectively. In those samples maintained in culture medium and assayed 5 days after irradiation, viability was reduced by 28-88%, with the least amount of deterioration in untreated and singly irradiated samples.

CONCLUSIONS: Functional fluorescent dyes combined with flow cytometric analysis successfully determines the effect of laser irradiation on the viability of reshaped cartilage.

Lasers Surg Med. 2003;32(4):286-93.

THE INFLUENCE OF LOW LEVEL INFRA RED LASER THERAPY ON THE REGENERATION OF CARTILAGE TISSUE

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This study concerns the influence of Laser treatment on the regeneration process of cartilage tissue. There is no need saying that the regeneration of cartilage tissue is a very big problem in rheumatic diseases for example. The lack of blood supply is one of the most important factors involved. Lots of previous publications give us proof of the regeneration capacities of Laser therapy (in wound healing, bone repair etc.)
In this study we have chosen to experiment on cartilage tissue of the ear of mice. We are aware of the fact that the elastic cartilage tissue of the ear is not totally comparable with the hyaline cartilage of articulations. For technical reasons however and because of the fact that the chondrocytes are comparable, we decided to use mice ears in our experiment. A 0,4 mm hole was drilled in both ears on 30 mice. The right ears remain untreated, while the left ears were treated daily with IR-Laser (904 nm) for 3 minutes. Macroscopical as well as histological evaluations were performed on the cartilage regeneration of both ears.

Our results show that after one day postsurgery no differences were found between the irradiated and the non-irradiated group. After the second day, only in the irradiated group there is a clear activation of the perichondrium. After four days, there is a significant ingrowth of the perichondrium into the drill hole in the experimental group and there is only an active perichondrium zone in our control group.

Lasers Surg Med. 2002; 31: 263-267.

Effect of low-power laser irradiation on cell growth and procollagen synthesis of cultured fibroblasts.

Pereira A, de Paula Eduardo C, Matson E et al

The cell growth and procollagen synthesis of cultured fibroblasts were studies by irradiation at energy densitites ranging from 3-5 J/cm2 over a period of 6 days. To simulate a situation of stress the cells were grown in a 2.5% FBS solution (10% being optimal). The laser was a 120 mW GaAs laser. Irradiation at 3 to 4 J/cm2 increased the cell numbers about threefold to sixfold, compared to control cultures. However, the effect was restricted to a small range of

densities, since 5 J/cm2 had no effect on cell growth. The energy density of 3 J/cm2 remarkably increased cell growth, with no effect on procollagen synthesis, as demonstrated by immunoprecipitation analysis.

Biomed Pharmacother. 2001 Mar;55(2):117-20.

Laser biostimulation of cartilage: in vitro evaluation.

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Abstract

An in vitro study was performed to evaluate the laser biostimulation effect on cartilage using a new gallium-aluminium-arsenic diode laser. Chondrocyte cultures were derived from rabbit and human cartilage. These cells were exposed to laser treatment for 5 days, using the following parameters: 300 joules, 1 watt, 100 (treatment A) or 300 (treatment B) hertz, pulsating emission for 10 minutes, under a sterile laminar flow. Control cultures (no treatment) received the same treatment with the laser device off. Cell viability was measured by MTT assay at the end of the laser treatment and then after 5 days. Neither rabbit nor human cultured chondrocytes showed any damage under a light microscope and immunostaining control following laser treatment. The MTT test results indicated a positive biostimulation effect on cell proliferation with respect to the control group. The increase in viability of irradiated chondrocytes was maintained for five days following the end of the laser treatment. The results obtained with the Ga-Al-As diode laser using the above tested parameters for in vitro biostimulation of cartilage tissues provide a basis for a rational approach to the experimental and clinical use of this device.

Lasers Surg Med. 2001;28(1):1-10.

Laser-mediated cartilage reshaping with feedback-controlled cryogen spray cooling: biophysical properties and viability.

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Abstract

BACKGROUND AND OBJECTIVE: Recent studies have indicated that chondrocyte viability decreases with prolonged or repeated laser irradiation. To optimize laser-mediated cartilage reshaping, the heating process must be finely controlled. In this study, we use high-

power Nd:YAG laser irradiation (lambda = 1.32 microm) combined with cryogen spray cooling (CSC) in an attempt to reshape porcine septal cartilage while enhancing chondrocyte viability.

STUDY DESIGN/MATERIALS AND METHODS: Chondrocyte viability was determined after high-power (50 W/cm2) Nd:YAG-mediated cartilage reshaping with and without cryogen spray cooling (CSC) and correlated with dynamic measurements of tissue optical and thermal properties.

RESULTS: After 1.5 to 2.0 seconds of laser exposure, characteristic changes in diffuse reflectance (indicating the onset of accelerated stress relaxation) was observed in both laser only and laser with CSC specimens. After 2 seconds of laser exposure, specimens in both groups retained the curved shape for up to 14 days. After one laser exposure, chondrocyte viability was 94.35 +/- 6.1% with CSC and 68.77 +/- 20.1% ($P < 0.05$) without CSC. After two laser exposures, a similar trend was observed with CSC (70.18 +/- 16.44%) opposed to without CSC (28 +/- 45%; $P < 0.05$).

CONCLUSION: CSC during high-power laser irradiation allows rapid heating while minimizing extreme front surface temperature elevations and axial thermal gradients. Laser irradiation with CSC can be used to effectively reshape cartilage tissue with the additional advantage of increasing chondrocyte viability.

Artif Cells Blood Substit Immobil Biotechnol. 2000 Mar;28(2):193-201.

Biostimulation of human chondrocytes with Ga-Al-As diode laser: ‘in vitro’ research.

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Abstract

The aim of this study was to verify the effects of laser therapy performed with Ga-Al-As Diode Lasers (780 nm, 2500 mW) on human cartilage cells in vitro. The cartilage sample used for the biostimulation treatment was taken from the right knee of a 19-year-old patient. After the chondrocytes were isolated and suspended for cultivation, the cultures were incubated for 10 days. The cultures were divided into four groups. Groups I, II, III were subject to biostimulation with the following laser parameters: 300 J, 1 W, 100 Hz, 10 min. exposure, pulsating emission; 300 J, 1 W, 300 Hz, 10 min. exposure, pulsating emission; and 300 J, 1 W, 500 Hz, 10 min. exposure, pulsating emission, respectively. Group IV did not receive any treatment. The laser biostimulation was conducted for five consecutive days. At the end of the treatment, the Calcium, Alkaline Phosphate, MTT tests and proteoglycan were performed to assess cell metabolism and toxicity level. The data showed good results in terms of cell viability and levels of Ca and Alkaline Phosphate in the groups treated with laser biostimulation compared to the untreated group. The results obtained confirm our previous positive in vitro results that the Ga-Al-As Laser provides biostimulation without cell damage.

Photochem Photobiol. 2000 Feb;71(2):218-24.

Proteoglycan synthesis in porcine nasal cartilage grafts following Nd:YAG (lambda = 1.32 microns) laser-mediated reshaping.

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Abstract

Mechanically deformed morphologic cartilage grafts undergo temperature-dependent stress relaxation during sustained laser irradiation resulting in stable shape changes. In this study, porcine nasal septal cartilage specimens were evaluated for viability by measuring the incorporation of Na2(35)SO4 into proteoglycan (PTG) macromolecules in whole tissue culture following laser-mediated reshaping. Synthesis rates of PTG were determined by scintillation counting lyophilized specimens and normalizing these values by total protein content. Positive controls were established by inducing chondrocyte apoptosis using prolonged exposure to nitric oxide (NO). In chondrocytes, apoptosis induced using NO resulted in significantly lower PTG synthesis rates compared to untreated native specimens. Cartilage specimens were irradiated with light emitted from a Nd:YAG laser (25 W/cm2, lambda = 1.32 microns) while recording simultaneously radiometric surface temperature, internal stress and back-scattered light intensity from a probe laser. Each specimen received one, two or three sequential laser exposures. The duration of each exposure was determined from real-time measurements of characteristic changes in back-scattered light intensity that correlate with accelerated stress relaxation. A 5 min time interval between each laser exposures allowed the cartilage specimen to return to thermal equilibrium. Average PTG synthesis rates decreased with successive laser exposures, though these were always higher than baseline rates established for NO-treated tissues, suggesting that laser-mediated cartilage reshaping acutely does not eliminate the entire population of viable chondrocytes. The reduction in PTG synthesis is correlated with the time-temperature-dependent heating profile created during laser irradiation, supporting our hypothesis that careful monitoring of laser dosimetry is required to ensure chondrocyte viability.

Acta Biomed Ateneo Parmense. 1999;70(3-4):43-7.

Cartilage cell stimulation with low-power laser: experimental assessment

[Article in Italian]

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Abstract

The aim of this study was to verify the effects of laser therapy performed with Ga-Al-As diode laser (780 nm, 2500 mW) on cartilage cells in vitro. The cartilage sample used for biostimulation was taken from the knee of an adult patient. The cultures were divided into four groups: Groups I, II, III were subjected to biostimulation with different laser parameters; Group IV did not received any treatment. The laser biostimulation was conducted for five consecutive days. At the end of the treatment, cell count and MTT tests were performed to assess cell metabolism. The data showed good results in terms of cell viability in the groups treated with laser biostimulation compared to the untreated group. The results obtained with the use of this new low-power diode laser Ga-Al-As device in the biostimulation of the cartilage tissue, permits us to consider the use of this device clinically.

Wound Repair Regen. 1999 Nov-Dec;7(6):518-27.

Matrix remodeling in healing rabbit Achilles tendon.

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Abstract

Biochemical, biomechanical and ultrastructural properties of the connective tissue matrix were investigated during the early remodeling phase of tissue repair in experimentally tenotomized and repaired rabbit Achilles tendons. Sterile surgical tenotomy was performed on the right Achilles tendons of 14 rabbits and allowed to heal for 15 days. The animals were euthanized and the Achilles tendons excised from both limbs. The left contralateral Achilles tendon of each rabbit was used as a control in the experiments. Prior to biochemical analysis, both intact and healing tendons were tested for their biomechanical integrity. The results revealed that the healing tendons had regained some of their physicochemical characteristics, but differed significantly from the intact left tendons. The healing tendons regained 48% tensile strength, 30% energy absorption, 20% tensile stress, and 14% Young's modulus of elasticity of intact tendons. In contrast, biochemical analysis showed that the healing tendons had 80% of the collagen and 60% of the collagen crosslinks (hydroxypyridinium) of normal tendons. Sequential extraction of collagen from the tissues yielded more soluble collagen in the healing tendons than intact tendons, suggesting either an increase in collagen synthesis and/or enhanced resorption of mature collagen in healing tendons compared to intact tendons. Electron microscopic studies revealed remarkable differences in the ultrastructure between intact and healing tendons. These observations could explain, in part, the connective tissue response to healing during the early phases of tissue remodeling.

Artif Cells Blood Substit Immobil Biotechnol. 1998 Jul;26(4):437-9.

In vitro experimental research of rabbit condrocytes biostimulation with diode laser Ga-Al-As: a preliminary study.

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Abstract

The scope of our study was to verify the effects of a new diode laser device with active material composed of Gallium, Aluminum and Arsenic (Ga-Al-As) configured as MOCVD (780 nm., 3000 mW) for the biostimulation of the cartilage cells in vitro. The condrocytes cells, withdrawn from the cartilage of the medial condyle of the femur of the rabbit, were cultivated, incubated and subject to biostimulation treatment with the laser. The condrocytes cells were placed in 24 Petri dishes at the concentration of 0.25 x 10(5)/ml and divided into 4 groups: 3 group (I, II, III) were treated with the laser and the fourth group (IV) was used as the control group. At the end of the treatment, all four groups, were evaluated with a MTT test and a cell count of the condrocytes cells. Group III (300 J, 1 Watt, 300 Hz, 10′ of exposure time with a pulsating emission) provided the best results in terms of cell viability (MTT test) and for the number of cells found in the dishes when compared to the other treated groups and the control group. The results obtained with the use of this new diode laser Ga-Al-As device in the biostimulation of the cartilage tissue, permits us to consider the use of this device clinically.

Med Sci Sports Exerc. 1998 Jun;30(6):794-800.

Biochemistry and biomechanics of healing tendon: Part II. Effects of combined laser therapy and electrical stimulation.

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Abstract

PURPOSE: In previous studies we demonstrated that early mechanical loading and laser photo-stimulation independently promoted tendon healing. Thus, we tested the hypothesis that a combination of laser phototherapy and mechanical load would further accelerate healing of experimentally tenotomized and repaired rabbit Achilles tendons.

METHODS: Following surgical tenotomy and repair, the tendons of experimental and control rabbits were immobilized in polyurethane casts for 5 d. The repaired tendons of experimental rabbits received mechanical load via electrical stimulation-induced contraction of the triceps surae for 5 d. In addition, experimental tendons were treated with daily doses of 1 J.cm-2 low intensity helium-neon laser throughout the 14-d experimental period.

RESULTS: The combination of laser photostimulation and mechanical load increased the maximal stress, maximal strain, and Young's modulus of elasticity of the tendons 30, 13, and 33%, respectively. However, MANOVA revealed no statistically significant differences in these biomechanical indices of repair of control and experimental tendons. Biochemical assays showed a 32% increase in collagen levels ($P < 0.05$) and an 11% decrease in mature cross-links in experimental tendons compared with that in controls ($P > 0.05$). Electron microscopy and computer morphometry revealed no significant differences in the morphometry of the collagen fibers and no visible differences in the ultrastructure of cellular and matrical components of control and experimental tendons.

CONCLUSIONS: These findings indicate that the combination of laser photostimulation and early mechanical loading of tendons increased collagen production, with marginal biomechanical effects on repaired tendons.

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Laser photostimulation of collagen production in healing rabbit Achilles tendons.

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Abstract

BACKGROUND AND OBJECTIVE: Low energy laser photostimulation at certain wavelengths can enhance tissue repair by releasing growth factors from fibroblasts and stimulate the healing process. This study was designed to evaluate the influence of laser photostimulation on collagen production in experimentally tenotomized and repaired rabbit Achilles tendons.

STUDY DESIGN/MATERIALS AND METHODS: A total of 24 male New Zealand rabbits, ages 10-12 weeks, were used. Following tenotomy and repair, the surgical hind limbs of the rabbits were immobilized in customized polyurethane casts. The experimental animals were treated with a 632.8 nm He:Ne laser daily at 1.0 J cm(-2) for 14 days. Control animals were sham treated with the laser head. On the fifth day after repair, the casts were removed to allow the animals to bear weight on the lower extremity. The animals were euthanized on the 15th postoperative day, then, the Achilles tendons were excised, processed and analyzed.

RESULTS: Biochemical analyses of the tendons revealed a 26% increase in collagen concentration with laser photostimulation indicating a more rapid healing process in treated tendons compared to controls. Sequential extractions of collagen from regenerating tissues revealed that the laser photostimulated tendons had 32% and 33% greater concentrations of neutral salt soluble collagen and insoluble collagen, respectively, than control tendons suggesting an accelerated production of collagen with laser photostimulation. A significant decrease (9%) in pepsin soluble collagen was observed in laser-treated tendons compared to controls. There were no statistically significant differences recorded in the concentrations of hydroxypyridinium crosslinks and acid soluble collagen between treated and control tendons.

CONCLUSION: This study of laser photostimulation on tendon healing in rabbits suggests that such therapy facilitates collagen production in a manner that enhances tendon healing.

Am J Phys Med Rehabil. 1997 Jul-Aug;76(4):288-96.

Combined ultrasound, electrical stimulation, and laser promote collagen synthesis with moderate changes in tendon biomechanics.

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Abstract

The biomechanical, biochemical, and ultrastructural effects of a multitherapeutic protocol were studied using regenerating rabbit Achilles tendons. The multitherapeutic protocol was composed of low-intensity Ga:As laser photostimulation, low intensity ultrasound, and electrical stimulation. Achilles tendons of 63 male New Zealand rabbits were tenotomized, sutured, immobilized, and subjected to the multitherapeutic protocol for five days, after which casts were removed and the therapy was continued for nine more days without electrical stimulation. The tendons were excised and compared with control tendons. Multitherapy treatment produced a 14% increase in maximal strength, a 42% increase in load-at-break, a 20% increase in maximal stress, a 45% increase in stress-at-break, a 21% increase in maximal strain, and a 14% increase in strain-at-break. Similarly, multitherapy treatment was associated with an increase in Young's modulus of elasticity of 31%, an increase in energy absorption at maximum load of 9%, and an increase in energy absorption at load-at-break of 11%. Biochemical analysis of the tendons showed an increase of 23% in the total amount of collagen in the multitherapy-treated tendons, with fewer mature crosslinks (decrease of 6%). Electron micrographs revealed no ultrastructural or morphologic changes in the tendon fibroblasts or in the extracellular matrix. The improvements measured in tendons receiving multitherapy were consistent but less remarkable compared with our earlier works with single modality protocols. The results warrant the hypothesis that the beneficial effects of ultrasound and laser photostimulation on tendon healing may counteract one another when applied simultaneously.

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Laser's effect on bone and cartilage change induced by joint immobilization: an experiment with animal model.

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OBJECTIVE: Influence of low-level (810 nm, Ga-Al-As semiconductor) laser on bone and cartilage during joint immobilization was examined with rats' knee model. MATERIALS AND METHODS: The hind limbs of 42 young Wistar rats were operated on in order to immobilize the knee joint. One week after operation they were assigned to three groups; irradiance 3.9 W/cm2, 5.8 W/cm2, and sham treatment. After 6 times of treatment for another 2 weeks both hind legs were prepared for 1) indentation of the articular surface of the knee (stiffness and loss tangent), and for 2) dual energy X-ray absorptiometry (bone mineral density) of the focused regions. RESULTS AND CONCLUSIONS: The indentation test revealed preservation of articular cartilage stiffness with 3.9 and 5.8 W/cm2 therapy. Soft laser treatment has a possibility for prevention of biomechanical changes by immobilization.

The Biological Effects of Laser Therapy and Other physical Modalities on Connective Tissue Repair Processes

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Connective tissue injuries, such as tendon rupture and ligamentous strains, are common. Unlike most soft tissues that require 7-10 days to heal, primary healing of tendons and other dense connective tissues take as much as 6 – 8 weeks during which they are inevitably protected in immobilization casts to avoid re-injury. Such long periods of immobilization impair functional rehabilitation and predispose a multitude of complications that could be minimized if healing is quickened and the duration of cast immobilization reduced. In separate studies, we tested the hypothesis that early function, ultrasound, 632.8 nm He-Ne laser, and 904 nm Ga-As laser, when used singly or in combination, promote healing of experimentally severed and repaired rabbit Achilles tendons as evidenced by biochemical, biomechanical, and morphological indices of healing. Our results demonstrate that: (1) appropriate doses of each modality, i.e., early functional activities, ultrasound, He-Ne and Ga-As laser therapy augment collagen synthesis, modulate maturation of newly synthesized collagen, and overall, enhance the biomechanical characteristics of the repaired tendons. (2) Combinations of either of the two lasers with early function and either ultrasound or electrical

stimulation further promote collagen synthesis when compared to functional activities alone. However, the biomechanical effects measured in tendons receiving the multi-therapy were similar, i.e., not better than the earlier single modality trials. Although tissue repair processes in humans may differ from that of rabbits, these findings suggest that human cases of connective tissue injuries, e.g., Achilles tendon rupture, may benefit from appropriate doses of He-Ne laser, Ga-As laser, and other therapeutic modalities, when used singly or in combination. Our recent meta-analysis of the laser therapy literature further corroborate these findings.