



Effects of Light on Osteoarthritis and Cartilage Repair

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Cartilage

Cartilage is composed of collagenous fibers and/or elastic fibers, and cells called chondrocytes, all of which are embedded in a firm gel-like ground substance called the matrix. Cartilage is avascular (contains no blood vessels) and nutrients are diffused through the matrix. Cartilage serves several functions, including providing a framework upon which bone deposition can begin and supplying smooth surfaces for the movement of articulating bones. There are three main types of cartilage: hyaline, elastic and fibrocartilage. Within articular cartilage, Hyaline and Fibrocartilage are the most important.

Types of Cartilage

Hyaline cartilage is the most abundant type of cartilage. The name hyaline is derived from the Greek word *hyalos*, meaning glass. This refers to the translucent matrix or ground substance. It is avascular hyaline cartilage that is made predominantly of type II collagen. Hyaline cartilage is found lining bones in joints (articular cartilage or, commonly, gristle). It can withstand tremendous compressive force, needed in a weight-bearing joint.

Fibrocartilage (also called white cartilage) is a specialized type of cartilage found in areas requiring tough support or great tensile strength, such as intervertebral discs and at sites connecting tendons or ligaments to bones (e.g., meniscus). There is rarely any clear line of demarcation between fibrocartilage and the neighboring hyaline cartilage or connective tissue. In addition to the type II collagen found in hyaline and elastic cartilage, fibrocartilage contains type I collagen that forms fiber bundles seen under the light microscope. When the hyaline cartilage at the end of long bones such as the femur is damaged, it is often replaced with fibrocartilage, which does not withstand weight-bearing forces as well.

Cartilage is composed of 4% chondrocytes and 96% extracellular matrix. Extracellular matrix is composed of:

- Type II collagen, a major support structure (Types I and III also present in smaller amounts)
- Proteoglycans, long fibrous chains, chiefly aggrecan. These are configured as globules, encased in the matrix by a mesh-like limiting lattice of Type II collagen. They are hydrophilic (absorbing 30 to 50 times their dry weight) and continually expand –contained by the lattice network of Type II collagen –to provide the shock-absorbing qualities of cartilage.
- Glycosaminoglycan chains, composed of keratin sulfate and chondroitin sulfate.
- Interstitial fluid, containing chiefly water and a host of proteins.

Chondrocytes balance the breakdown and repair processes of cartilage. They differ from other animal cells in that they have no blood supply, no lymphatics, and lack access to nerves. Joint movement and compression cause flows within the matrix that move diffused nutrients and stimulates the breakdown and repair factors.

Cartilage Metabolism: Promotional and Degradation Factors

As with many body systems, cartilage is maintained by a balance of tissue promotion and degradation factors. Promotional factors include Aggrecan and collagen formation and “tissue inhibitor of metalloproteinases” (TIMP). Other pro-cartilage factors include bone growth factors, which have a role in the preservation of the cartilage matrix. These include bone morphogenetic proteins, insulin-like growth factors, hepatocyte growth factor, basic fibroblast growth factor, transforming growth factor beta, and Stress Proteins (also known as Heat Shock Proteins). What these pro-cartilage factors have in common is that they operate directly on stem cells, which is the mechanism for cartilage repair.

Degradation factors in cartilage include matrix metalloproteinase (MMP) enzymes, aggrecanases, collagenases, activators of MMPs and nitric oxide (*inducible form*). Within the cartilage matrix,

inducible nitric oxide plays an opposite role to that of endothelial nitric oxide found in well-vascularized tissues where it functions as a critical signaling factor for tissue repair. This contradistinction can be seen in other organ systems¹.

Inducible vs Endothelial Nitric Oxide: Effects in Cartilage

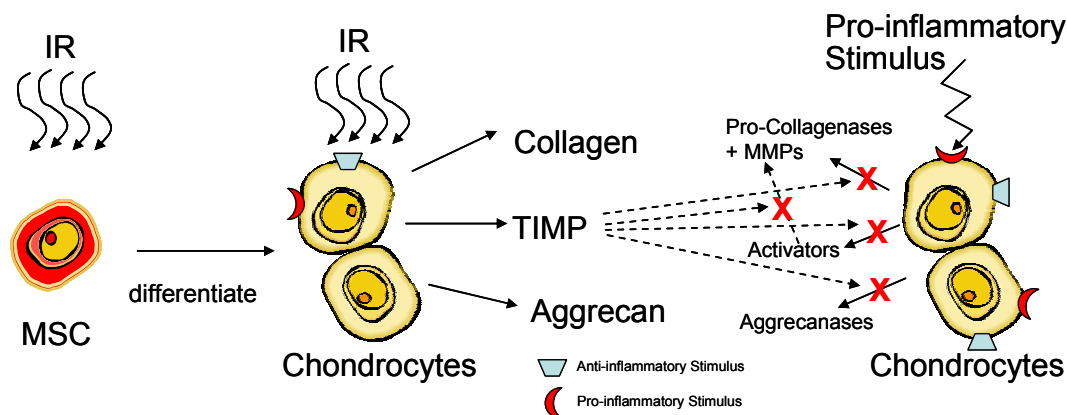
Zhou, et al, examined renal glomerular thrombotic microangiopathy (TMA) and associated levels of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS). The investigators found administration of *E. coli* endotoxin leads to a sustained fall in renal eNOS expression and concomitant rise in iNOS expression both in vivo and in vitro. The associated decline in intrarenal endothelial NO production/availability may result in renal vasoconstriction and a hypercoagulative state, which may contribute to the pathogenesis of endotoxin-induced TMA.²

Cartilage contains mostly the iNOS isoform so it only produces HIGH, damaging levels of NO in disease states, and only when there is preceding injury or infection. The goal would be to suppress iNOS activity. Low levels of NO from sodium nitro prusside or other "physiologic" nitric oxide donors suppress the activity of iNOS. It is believed that infrared light releases NO from endothelial cells and RBCs at the site of application. And if this is a damaged joint, the iNOS activity sustaining the production of very high levels of NO, will be reduced through the local inhibitory action of the small increase in physiologic NO concentration (some 100 to 1000 times less than that produced by iNOS) from endothelial cells and RBC. Further cartilage damage will be minimized, swelling/inflammation will be reduced and pain will be reduced.

Cartilage Repair Arises from Stem Cells

Studies also indicate that low energy light has a direct stimulative effect on mesenchymal stem cells, causing them (in the cartilage environment) to differentiate into collagen (chiefly Type II). The chief pathway is via direct infrared light stimulation of cytochrome C oxidase in the mitochondria which results in increased metabolism, which leads to signal transduction to other parts of the cell.

Observation shows a fundamental lack of repair in articular cartilage where the damage does not penetrate the subchondral bone. This indicates the importance of marrow components in the repair of the articular cartilage. In adult animals, there is an inability of articular cartilage chondrocytes to heal chondral defects, but if the damage extends beyond the subchondral bone, a repair process ensues in which mesenchymal progenitor cells (MSCs) migrate into the injured site and undergo chondrogenic differentiation. However, analysis of animal models and human biopsy samples indicates that fibrocartilage, rather than true articular (hyaline) cartilage is the predominant tissue synthesized. A number of approaches are under investigation to determine how to stimulate hyaline formation rather than fibrocartilage. These include cell based implants of culture expanded progenitor cells from various sources and, as described in this paper, use of red and infrared irradiation of mesenchymal stem cells *in-vivo*.





Growth of Cartilage

Two types of growth can occur in cartilage: appositional and interstitial. Appositional growth results in the increase of the diameter or thickness of the cartilage. The new cells derive from the perichondrium and occur on the surface of the cartilage model. (The perichondrium is a layer of dense connective tissue which surrounds cartilage. It consists of two separate layers: an outer fibrous layer and inner chondrogenic layer. The fibrous layer contains fibroblasts, which produce collagenous fibers. The chondrogenic layer remains undifferentiated and can form chondroblasts or chondrocytes.) Interstitial growth results in an increase of cartilage mass and occurs from within. Chondrocytes undergo mitosis within their lacuna, but remain imprisoned in the matrix, which results in clusters of cells called isogenous groups.

Diseases of Cartilage

There are several diseases which can affect the cartilage. Chondrodystrophies are a group of diseases characterized by disturbance of growth and subsequent ossification of cartilage. Arthritis is a condition where the cartilage covering bones in joints (articular cartilage) is degraded, resulting in limitation of movement and pain. Arthritis may occur due to trauma or age-related "wear and tear" (osteoarthritis) or due to an autoimmune reaction against joint components (rheumatoid arthritis).

Sources of Light: Lasers and Light Emitting Diodes (LEDs)

Much of the early work and indeed much of the available data on light therapies is based on laser light. In recent years, LEDs have become a popular choice for delivering light therapy based on safety, cost and large area of coverage. A number of prominent researchers have examined the question of whether there is a clinical difference in light sources. They have concluded that source of light is not as important as wavelength, energy dose and frequency.

Dr. Kendric C. Smith at the Department of Radiation Oncology, Stanford University School of Medicine, concludes in an article entitled The Photobiological Effect of Low Level Laser Radiation Therapy (Laser Therapy, Vol. 3, No. 1, Jan - Mar 1991) that, "1. Lasers are just convenient machines that produce radiation. 2. It is the radiation that produces the photobiological and/or photophysical effects and therapeutic gains, not the machines. 3. Radiation must be absorbed to produce a chemical or physical change, which results in a biological response."³

In a study entitled Low-Energy Laser Therapy: Controversies and New Research Findings, Jeffrey R. Basford, M.D. of the Mayo Clinic's Department of Physical Medicine and Rehabilitation, suggests that the coherent aspect of laser may not be the source of its therapeutic effect. He states "firstly, the stimulating effects (from therapeutic light) are reported following irradiation with non-laser sources and secondly, tissue scattering, as well as fiber optic delivery systems used in any experiments rapidly degrade coherency. Thus any effects produced by low-energy lasers may be due to the effects of light in general and not to the unique properties of lasers. In this view, laser therapy is really a form of light therapy, and lasers are important in that they are convenient sources of intense light at wavelengths that stimulate specific physiological functions."⁴

Bjordan and colleagues examined numerous studies, concluding as follows: "The scarcity of literature on LED is responsible for consultation of literature originating from LLL (Low Level Laser) studies but it may be wondered if this literature is representative for that purpose. As in the early days of LLL therapy, the stimulating effects upon biological objects were explained by its coherence while the beam emitted by LED's on the contrary produces incoherent light. Though the findings of some scientists nowadays (show) that the coherence of the light beam is not responsible for the effects of LLL therapy. Given that the cardinal difference between LED and LLL therapy, coherence, is not of remarkable importance in providing biological response in cellular monolayers, one may consult literature from LLL studies to refer to in this LED studies."⁵

Biology of Cartilage Repair

I. Cartilage repair arises from Mesenchymal cells, which differentiate into Cartilaginous cells and Extracellular matrix

The following study demonstrates that cartilage repair is achieved through proliferation and differentiation of mesenchymal (primordial, undifferentiated) cells, not from proliferation of extant cartilage chondrocytes.

Cell origin and differentiation in the repair of full-thickness defects of articular cartilage.

Shapiro F, Koide S, Glimcher MJ. J Bone Joint Surg Am. 1993 Apr;75(4):532-53. Links

Department of Orthopaedic Surgery, Children's Hospital, Boston, Massachusetts 02115.

The origin and differentiation of cells in the repair of three-millimeter-diameter, cylindrical, full-thickness drilled defects of articular cartilage were studied histologically in New Zealand White rabbits. The animals were allowed to move freely after the operation. Three hundred and sixty-four individual defects from 122 animals were examined as long as forty-eight weeks postoperatively. In the first few days, fibrinous arcades were established across the defect, from surface edge to surface edge, and this served to orient mesenchymal cell ingrowth along the long axes. The first evidence of synthesis of a cartilage extracellular matrix, as defined by safranin-O staining, appeared at ten days. At two weeks, cartilage was present immediately beneath the surface of collagenous tissue that was rich in flattened fibrocartilaginous cells in virtually all specimens. At three weeks, the sites of almost all of the defects had a well demarcated layer of cartilage containing chondrocytes. An essentially complete repopulation of the defects occurred at six, eight, ten, and twelve weeks, with progressive differentiation of cells to chondroblasts, chondrocytes, and osteoblasts and synthesis of cartilage and bone matrices in their appropriate locations. At twenty-four weeks, both the tidemark and the compact lamellar subchondral bone plate had been re-established. The cancellous woven bone that had formed initially in the depths of the defect was replaced by lamellar, coarse cancellous bone. Autoradiography after labeling with 3H-thymidine and 3H-cytidine demonstrated that chondrocytes from the residual adjacent articular cartilage did not participate in the repopulation of the defect. The repair was mediated wholly by the proliferation and differentiation of mesenchymal cells of the marrow. Intra-articular injections of 3H-thymidine seven days after the operation clearly labeled this mesenchymal cell pool. The label, initially taken up by undifferentiated mesenchymal cells, progressively appeared in fibroblasts, osteoblasts, articular chondroblasts, and chondrocytes, indicating their origin from the primitive mesenchymal cells of the marrow. Early traces of degeneration of the cartilage matrix were seen in many defects at twelve to twenty weeks, with the prevalence and intensity of the degeneration increasing at twenty-four, thirty-six, and forty-eight weeks. Polarized light microscopy demonstrated failure of the newly synthesized repair matrix to become adherent to, and integrated with, the cartilage immediately adjacent to the drill-hole, even when light microscopy had shown apparent continuity of the tissue. In many instances, a clear gap was seen between repair and residual cartilage.

The following study, based on the authors' 10 years of research in cartilaginous tissue engineering, reinforces the concept that articular cartilage repair arises from primitive mesenchymal cells rather than from more mature chondrocytes.

Cartilage Tissue Engineering: Current Limitations and Solutions.

Association of Bone and Joint Surgeons Workshop Supplement

Grande, Daniel A. PhD; Breitbart, Arnold S. MD *; Mason, James PhD **; Paulino, Carl MD; Laser, Jordan BS; Schwartz, Robert E. MD

Clinical Orthopaedics & Related Research. 367 SUPPLEMENT:S176-S185, October 1999.

Articular cartilage repair remains one of the most intensely studied orthopaedic topics. To date the field of tissue engineering has ushered in new methodologies for the treatment of cartilage defects. The authors' 10-year experience using principles of tissue engineering applied to resurfacing of cartilage defects is reported. Which cell type to use, chondrocytes versus chondroprogenitor cells, and their inherent advantages and disadvantages are discussed. Chondrocytes initially were used as the preferred cell type but were shown to have long term disadvantages in models used by the authors. Mesenchymal stem cells can be used effectively to overcome the limitations experienced with the use of differentiated chondrocytes. The use of mesenchymal stem cells as platforms for retroviral transduction of genes useful in cartilage repair introduces the concept of gene modified tissue engineering. The fundamental conditions for promoting and conducting a viable cartilage repair tissue, regardless of which cell type is used, also were studied. Placement of a synthetic porous biodegradable polymer scaffold was found to be a requirement for achieving an organized repair capable of functionally resurfacing a cartilage defect. A new modular device for intraarticular fixation of various graft composites has been developed. This new cartilage repair device is composed of bioabsorbable polymers and is capable of being delivered by the arthroscope.

The following study points out that mesenchymal stem cells (MSC) proliferate, differentiate, engraft and interface well with adjacent tissues (normal cartilage, bone) and form hyaline-like tissue. This suggests a pathway by which Light Therapy might promote formation of hyaline cartilage –namely, by stimulating MSCs to differentiate and proliferate into chondrocytes yielding Type II collagen resulting in hyaline formation.

Repair of Large Articular Cartilage Defects with Implants of Autologous Mesenchymal Stem Cells Seeded into β -Tricalcium Phosphate in a Sheep Model

Tissue Engineering, Nov 2004, Vol. 10, No. 11-12: 1818 -1829

Ximin Guo, M.D., Ph.D., Changyong Wang, M.D., Ph.D., Yufu Zhang, M.D., Renyun Xia, M.D., Min Hu, M.D., Ph.D., Cuimi Duan, Qiang Zhao, Lingzhi Dong, Jianxi Lu, M.D, Ph.D., Ying Qing Song, M.D., Ph.D.

Tissue engineering has long been investigated to repair articular cartilage defects. Successful reports have usually involved the seeding of autologous chondrocytes into polymers. Problems arise because of the scarcity of cartilage tissue biopsy material, and because the in vitro expansion of chondrocytes is difficult; to some extent, these problems limit the clinical application of this promising method. Bone marrow-derived mesenchymal stem cells (MSCs) have been proved a potential cell source because of their in vitro proliferation ability and multilineage differentiation capacity. However, in vitro differentiation will lead to high cost and always results in decreased cell viability. In this study we seeded culture-expanded autologous MSCs into bioceramic scaffold- β -tricalcium phosphate (β -TCP) in an attempt to repair articular cartilage defects (8 mm in diameter and 4 mm in depth) in a sheep model. Twenty-four weeks later, the defects were resurfaced with hyaline-like tissue and an ideal interface between the engineered cartilage, the adjacent normal cartilage, and the underlying bone was observed. From 12 to 24 weeks postimplantation, modification of neocartilage was obvious in the rearrangement of surface cartilage and the increase in glycosaminoglycan level. These findings suggest that it is feasible to repair articular cartilage defects with implants generated by seeding autologous MSCs, without in vitro differentiation, into β -TCP. This approach provides great potential for clinical applications.

This study further demonstrates the ability of non-differentiated mesenchymal cells to expand and differentiate into a number of different joint space cells, as influenced by physical contact with native mature cells (site dependent differentiation). In this case, the MSC cells are transplanted into degenerative discs and differentiate into cells expressing a number of key cell-associated matrix molecules.

Differentiation of Mesenchymal Stem Cells Transplanted to a Rabbit Degenerative Disc Model: Potential and Limitations for Stem Cell Therapy in Disc Regeneration.

Sakai, Daisuke MD *+; Mochida, Joji MD *+; Iwashina, Toru MD *+; Watanabe, Takuya MD *+; Nakai, Tomoko *+; Ando, Kiyoshi MD +; Hotta, Tomomitsu MD +
Spine. 30(21):2379-2387, November 1, 2005.

Study Design. An in vivo study to assess the differentiation status of mesenchymal stem cells (MSCs) transplanted to the nucleus pulposus of degenerative discs in a rabbit model.

Objectives. To evaluate the fate of MSCs transplanted to the nucleus pulposus of degenerative discs in a rabbit and to determine whether they are a suitable alternative for cell transplantation therapy for disc degeneration.

Summary of Background Data. Although MSCs have been proposed as candidate donor cells for transplantation to treat intervertebral disc degeneration, their differentiation after transplantation has not been adequately investigated.

Methods. Autologous MSCs, labeled with green fluorescent protein, were transplanted into mature rabbits. Consecutive counts of transplanted MSCs in the nucleus area were performed for 48 weeks after transplantation. Differentiation of transplanted cells was determined by immunohistochemical analysis. The proteoglycan content of discs was measured quantitatively using a dimethylmethylene blue assay and mRNA expression of Type I and II collagen, aggrecan and versican was measured semi-quantitatively using reverse transcription polymerase chain reaction.

Results. Many cells that were positive for green fluorescent protein (GFP) were observed in the nucleus pulposus of cell-transplanted rabbit discs 2 weeks after transplantation. Their number increased significantly by 48 weeks. Some GFP-positive cells were positive for cell-associated matrix molecules, such as Type II collagen, keratan sulfate, chondroitin sulfate, aggrecan, and the nucleus pulposus phenotypic markers, hypoxia inducible factor 1 alpha, glutamine transporter 1, and matrix metalloproteinase 2. MSCs did not show significant expression of these molecules before transplantation. Biochemical and gene expression analyses showed significant restoration of total proteoglycan content and matrix-related genes compared with nontransplanted discs.

Conclusions. MSCs transplanted to degenerative discs in rabbits proliferated and differentiated into cells expressing some of the major phenotypic characteristics of nucleus pulposus cells, suggesting that these MSCs may have undergone site-dependent differentiation. Further studies are needed to evaluate their functional role.

A great deal of current clinical research into OA treatment is focused on tissue engineering. In particular, techniques are being developed to grow chondrocytes on bio-matrixes and transplant into cartilage lesions in OA-affected joints.

The following study suggests that the in-vivo source of the mesenchymal, or progenitor cells, is from the surface of the articular cartilage itself. If light therapy stimulates MSC differentiation and proliferation, this would be the target population.

The surface of articular cartilage contains a progenitor cell population

Gary P. Dowthwaite, Joanna C. Bishop, Samantha N. Redman, Ilyas M. Khan, Paul Rooney, Darrell J. R. Evans, Laura Houghton, Zubeyde Bayram, Sam Boyer, Brian Thomson, Michael S. Wolfe and Charles W. Archer. Journal of Cell Science 117, 889-897 (2004)

It is becoming increasingly apparent that articular cartilage growth is achieved by apposition from the articular surface. For such a mechanism to occur, a population of stem/progenitor cells must reside within the articular cartilage to provide transit amplifying progeny for growth. Here, we report on the isolation of an articular cartilage progenitor cell from the surface zone of articular cartilage using differential adhesion to fibronectin. This population of cells exhibits high affinity for

fibronectin, possesses a high colony-forming efficiency and expresses the cell fate selector gene Notch 1. Inhibition of Notch signaling abolishes colony forming ability whilst activated Notch rescues this inhibition. The progenitor population also exhibits phenotypic plasticity in its differentiation pathway in an embryonic chick tracking system, such that chondroprogenitors can engraft into a variety of connective tissue types including bone, tendon and perimysium. The identification of a chondrocyte subpopulation with progenitor-like characteristics will allow for advances in our understanding of both cartilage growth and maintenance as well as provide novel solutions to articular cartilage repair.

Chondrocyte type and morphology varies depending on the zone of cartilage in which it is found. Chondrocytes from each zone produce a distinct set of matrix components. Stimulation of these various chondrocytes will yield different components, from surface zone proteoglycan, to midzone type II collagen fibers and the high molecular weight aggregating proteoglycan aggrecan which form hyaline cartilage, to deep zone type X collagen.

Differentiation of chondrocytes across cartilage zones and the resultant matrix component synthesis. (Ibid)

Articular cartilage is an avascular, aneural tissue with a high matrix to cell volume ratio. The matrix comprises mainly type II collagen fibers and the high molecular weight aggregating proteoglycan aggrecan. The tissue is not, however, homogeneous with biochemical and morphological variations existing from the surface zone to the deeper calcified layer. The surface zone of the tissue is characterized by flattened, discoid cells that secrete surface zone proteoglycan (proteoglycan 4) (Schumacher et al., 1994). The mid zone of the tissue comprises rounded cells arranged in perpendicular columns and in addition to type II collagen and aggrecan, expresses cartilage intermediate layer protein (CILP) (Lorenzo et al., 1998). The deep zone and calcified zone chondrocytes express type X collagen and alkaline phosphatase (Schmid and Linsenmayer, 1985), and in the deep zone the chondrocytes are considerably larger than in the other zones.

Clearly, the differentiation and proliferation events occurring during the development of articular cartilage must, therefore, be strictly controlled both temporally and spatially in order for the distinct zonal architecture of the tissue to be established. Various studies have shown that the surface zone of articular cartilage is centrally involved in the regulation of tissue development and growth. Not only does the surface of articular cartilage play a major role in the morphogenesis of the diarthrodial joint via differential matrix synthesis (Ward et al., 1999), but the expression of many growth factors and their receptors at the articular surface (Archer et al., 1994 ; Hayes et al., 2001) suggest that this region represents an important signaling centre. In addition, it has been shown in vivo that the surface zone of articular cartilage is responsible for the appositional growth of articular cartilage and from these studies we hypothesized that the surface zone of articular cartilage contains a progenitor/stem cell population that allows for the appositional growth of the tissue (Hayes et al., 2001).

II. Effects of Light Therapies (red and infrared) on Mesenchymal Cells fated to cartilaginous cell differentiation

This study demonstrates stimulatory effects of low level light therapy on mesenchymal cells – in this instance, mesenchymal cell which differentiate into chondroblasts.

Biostimulation of bone marrow cells with a diode soft laser.

Dortbudak O, Haas R, Mallath-Pokorny G. Clin Oral Implants Res. 2000 Dec;11(6):540-5.

In recent years, the use of low-intensity red light in regeneration of soft tissue has been increasingly pursued. As far as hard tissue is concerned, the biostimulating effect of laser has already been demonstrated successfully in more rapid healing of tibial bone fractures in mice at a

dosage of 2.4 J. However, the effect of light of a low dose laser directly on osteoblasts has not been investigated yet. The aim of this study was to determine the effect of continuous wave diode laser irradiation on osteoblasts derived mesenchymal cells. Three groups of 10 cultures each were irradiated 3 times (days 3, 5, 7) with a pulsed diode soft laser with a wavelength of 690 nm for 60 s. Another 3 groups of 10 cultures each were used as control groups. A newly developed method employing the fluorescent antibiotic tetracycline was used to compare bone growth on these culture substrates after a period of 8, 12 and 16 days, respectively. It was found that all laser cultures demonstrated significantly more fluorescent bone deposits than the non-laser cultures. The difference was significant, as tested by the Tukey Test ($P < 0.0001$) in the cultures examined after 16 days. Hence it is concluded that irradiation with a pulsed diode soft laser has a biostimulating effect on osteoblasts in vitro, which might be used in osseointegration of dental implants.

The following study demonstrates biostimulatory effects of light on chondrocyte cell cultures.

Laser biostimulation of cartilage: in vitro evaluation

P. Torricelli, G. Giavaresi, M. Fini, G. A. Guzzardella, G. Morrone, A. Carpi and R. Giardino. Biomedicine & Pharmacotherapy Volume 55, Issue 2, March 2001, Pages 117-120.

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.

III. Other examples of Light-stimulated proliferation and differentiation of Mesenchymal cells

This study examines the effects of infrared and polarized light on primitive cell (myofibroblasts) proliferation and differentiation in the tissue healing process, using measures of morphologic and cytochemical expression. Light therapy is demonstrated to increase healing activities by these measures as compared to non-treated controls. Taken together with studies of light stimulation of progenitor cells resulting in differentiation and proliferation in a variety of tissue types (e.g., muscle, dermis, bone, cartilage), this suggests a general mechanism of action for light on progenitor cells.

Polarized Light (400–2000 nm) and Non-ablative Laser (685 nm): A Description of the Wound Healing Process Using Immunohistochemical Analysis

Dr. Antonio Luiz B. Pinheiro, Ph.D., Daniel Humberto Pozza, D.D.S., Marilia G. De Oliveira, Ph.D., Ruben Weissmann, Ph.D., Luciana Maria Pedreira Ramalho, Ph.D. Photomedicine and Laser Surgery, Oct 2005, Vol. 23, No. 5 : 485 -492

Objective: This study aimed to describe, through morphologic and cytochemical analysis, the healing process of wounds submitted (or not) to laser therapy ($\lambda 685$ nm) or polarized light ($\lambda 400$ –2000 nm).

Background Data: There are many reports on different effects of several types of phototherapies on the treatment of distinct conditions, amongst them, on wound healing. Laser therapy and the use of polarized light are still controversial despite successive reports on their positive effects on several biological processes.

Methods: Thirty male Wistar rats, approximately 4 months old, were used, and standardized excisional wounds were created on their dorsum. The wounds were irradiated in four equidistant points with laser light or illuminated with polarized light, both with doses of 20 or 40 J/cm². Group 1 acted as untreated controls. Animals were irradiated every 48 h during 7 days, starting immediately after surgery, and were humanely killed on the 8th post-operative day. Specimens were taken and routinely processed and stained with H&E, and for descriptive analysis of myofibroblasts and collagen fibers, the specimens were immunomarked by smooth muscle α -actin and picrosirius stain.

Results:

- 1) Control specimens showed the presence of ulceration, hyperemia, discrete edema, intense, and diffuse inflammation, collagen deposition was irregular, and myofibroblasts were seen parallel to the wound margins.
- 2) Wounds treated by laser therapy with a dose of 20 J/cm² showed mild hyperemia, inflammation varied from moderate to intense, the number of fibroblasts was large, and the distribution of collagen fibers was more regular.
- 3) Increasing the dose to 40 J/cm² evidenced exuberant neovascularization, severe hyperemia, moderate to severe inflammation, large collagen deposition, and fewer myofibroblasts.
- 4) On subjects illuminated with polarized light with a dose of 20 J/cm², mild to moderate hyperemia was detectable, and collagen matrix was expressive and unevenly distributed; a larger number of myofibroblasts was present and no re-epithelialization was seen.
- 5) Increasing the dose resulted in mild to moderate hyperemia, no re-epithelialization was seen, edema was discrete, and inflammation was moderate.

Conclusion: The use of 685-nm laser light or polarized light with a dose of 20 J/cm² resulted in increased collagen deposition and better organization on healing wounds, and the number of myofibroblast was increased when polarized light is used.

Low level light therapy has been shown to enhance quality of fibroblast cell cultures in terms of engraftment, colony forming efficiency and clonal growth rates.

Laser therapy accelerates initial attachment and subsequent behaviour of human oral fibroblasts cultured on titanium implant material: A scanning electron microscopic and histomorphometric analysis

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The aim of the study was to investigate the effect of low-level laser therapy (LLLT) on attachment and proliferation of human gingival fibroblasts (HGF) cultured on titanium implant material. HGF were exposed to gallium–aluminum–arsenide diode laser at dosages of 1.5 or 3 J/cm² and then cultured on commercially pure titanium discs. Cell profile areas were measured after 1, 3 and 24 h, using scanning electron microscopy and an automatic image analyzer. The results were expressed as percentage of attachment. In order to investigate the effect of LLLT on cellular growth after 8 and 10 days, HGF were cultured on titanium discs for 24 h and then exposed to laser irradiation on 3 consecutive days. Colony-forming efficiency (CFE) and clonal growth rates (CGR) were measured. Cell viability was determined by Hoechst and prodidium iodide staining.

Non-lased cultures served as controls. Morphologically, the cells spread well on all titanium surfaces, indicating good attachment by both irradiated and non-irradiated cells. Fibroblasts exposed to laser irradiation had significantly higher percentages of cell attachment than the non-exposed cells ($P < 0.05$). CFE and CGR were also enhanced for the irradiated cells ($P < 0.05$). Cell viability was high ($> 90\%$) in the irradiated and control groups, without significant differences. It is concluded that in vitro LLLT enhances the attachment and proliferation of HGF on titanium implant material.

Low energy red and infrared light has been shown to promote proliferation of precursor satellite cells, similar to precursor mesenchymal cells.

Skeletal muscle cell activation by low-energy laser irradiation: A role for the MAPK/ERK pathway

Gavriela Shefer 1, Uri Oron 2, Andrey Irintchev 3, Anton Wernig 3, Orna Halevy. Journal of Cellular Physiology Volume 187, Issue 1, Pages 73 – 80, 2001

Low-energy laser irradiation (LELI) has been shown to promote skeletal muscle regeneration in vivo and to activate skeletal muscle satellite cells, enhance their proliferation and inhibit differentiation in vitro. In the present study, LELI, as well as the addition of serum to serum-starved myoblasts, restored their proliferation, whereas myogenic differentiation remained low. LELI induced mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK) phosphorylation with no effect on its expression in serum-starved myoblasts. Moreover, a specific MAPK kinase inhibitor (PD098059) inhibited the LELI- and 10% serum-mediated ERK1/2 activation. However, LELI did not affect Jun N-terminal kinase (JNK) or p38 MAPK phosphorylation or protein expression. Whereas a 3-sec irradiation induced ERK1/2 phosphorylation, a 12-sec irradiation reduced it, again with no effect on JNK or p38. Moreover, LELI had distinct effects on receptor phosphorylation: it caused phosphorylation of the hepatocyte growth factor (HGF) receptor, previously shown to activate the MAPK/ERK pathway, whereas no effect was observed on tumor suppressor necrosis (TNF-) receptor which activates the p38 and JNK pathways. Therefore, by specifically activating MAPK/ERK, but not JNK and p38 MAPK enzymes, probably by specific receptor phosphorylation, LELI induces the activation and proliferation of quiescent satellite cells and delays their differentiation.

IV. Effects of Light Therapies on Osteoarthritis in Animal Models

1) Light Stimulated Cartilage Formation

The following three abstracts provide a model for cartilage degradation (through joint immobilization) and for repair (through light therapy). The effect of low level light therapy on in-vivo rabbit cartilage healing, marked by chondrocyte growth, is demonstrated in the second and third studies.

(1) Joint immobilization as a model for induced OA

Apoptosis and p53 expression in chondrocytes relate to degeneration in articular cartilage of immobilized knee joints.

Okazaki R, Sakai A, Ootsuyama A, Sakata T, Nakamura T, Norimura T. J Rheumatol. 2003 Mar;30(3):559-66.

OBJECTIVE: We have reported that articular cartilage showed early stage degeneration at 7 and 14 days after immobilization, moderate degeneration at 28 days, and severe degeneration at 42 days in rabbits. To test whether apoptosis occurs in association with p53 expression in chondrocytes during the process of articular cartilage degeneration, we investigated the degree of cartilage degeneration, the frequency of apoptotic cells, and the levels of p53 mRNA in rabbits

and mice after knee immobilization. **METHODS:** Right knees of male Japanese white rabbits were immobilized in full extension with fiberglass casts for up to 42 days. Similarly, right knees of male p53 wild-type [p53 (+/+)] and p53 null [p53 (-/-)] mice were immobilized in full extension with bandage tape for up to 84 days. Apoptotic cells were confirmed by TUNEL staining on the sections of knee joints. Total RNA of articular chondrocytes obtained from Day 0 or immobilized knees was analyzed semiquantitatively by RT-PCR using specific primers for p53. **RESULTS:** Articular cartilage degenerated after immobilization of p53 (+/+) mouse knees, but not after immobilization of p53 (-/-) knees. Apoptotic cells were observed in articular cartilage in the femur and tibia of rabbits and p53 (+/+) mice after immobilization. However, only a few apoptotic cells were observed at the same sites in p53 (-/-) mice. In RT-PCR analysis, the levels of p53 mRNA obtained from immobilized groups were significantly higher than those of Day 0 groups in rabbit and p53 (+/+) mouse knees. **CONCLUSION:** Apoptosis and p53 expression in chondrocytes relate to degeneration in articular cartilage of immobilized knee joints.

(2) Light irradiation of immobilized joints improves surface morphology of articular cartilage.

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

Bayat M, Ansari A, Hekmat H. Indian J Exp Biol. 2004 Sep;42(9):866-70.

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 neutralized adverse effects of immobilization on articular cartilage.

(3) Infrared irradiation of immobilized joints preserves biomechanical integrity of articular cartilage.

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

Masami Akai, MD, Mariko Usuba, RPT, Toru Maeshima, Yoshio Shirasaki, Shozo Yasuoka, MD. Lasers Surg. Med. 21:480-484, 1997.

Objective

Influence of low-level (810nm, Ga-Al-As semi-conductor) 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 3.9W/cm², 5.8W/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 5.8W/cm² therapy. Soft laser treatment has a possibility for prevention of biomechanical changes by immobilization.



Infrared irradiation of surgically induced lesions in articular cartilage resulted in defect healing with good cell and tissue morphology compared to untreated joints.

Low-Power Diode Laser Stimulation of Surgical Osteochondral Defects: Results after 24 Weeks

Gaetano Antonio Guzzardella ^{A1}, Domenico Tiganì ^{A2}, Paola Torricelli ^{A1}, Milena Fini ^{A1}, Lucia Martini ^{A1}, Gianfranco Morrone A1, Roberto Giardino A1

Artificial Cells, Blood Substitutes, and Biotechnology (formerly known as Artificial Cells, Blood Substitutes, and Immobilization Biotechnology) Volume 29, Number 3 Pages: 235 - 244 / 2001

SUMMARY The purpose of this study was to evaluate osteochondral lesions of the knee, treated intraoperatively with low-power laser stimulation, and assess results at 24 weeks. Surgery was performed under general anesthesia on six rabbits; a bilateral osteochondral lesion was created in the femoral medial condyles with a drill. All of the left lesions underwent immediate stimulation using the diode Ga-Al-As laser (780nm), whereas the right knees were left untreated as control group. After 24 weeks, the explants from the femoral condyles, either treated employing laser energy or left untreated, were examined histomorphometrically. Results obtained on the lased condyles showed good cell morphology and a regular aspect of the repaired osteocartilaginous tissue.

Infrared light at 904 nm wavelength accelerates cartilaginous tissue formation.

The Influence of Low Level Infra-Red Laser Therapy On The Regeneration Of Cartilage Tissue

P.Lievens , Ph.van der Veen, Laser Florence 2003; <http://www.laser.nu>

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 post surgery 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.

Additional models of cartilage formation as stimulated by Infrared Light at 904 nm wavelength show acceleration of growth and differences in tissue type in treated versus untreated.

The Regenerating Capacity of LLLT on Muscle Fibers and On Cartilage Tissue

van der Veen Ph, Lievens P Department of Rehabilitation Research Vrije Universiteit Brussel, Brussels, Belgium. The Journal of Laser Therapy Millennium Edition Volumes 12 & 13; pg 27; 2000.

Introduction: This study concerns the influence of laser treatment on the regeneration process of cartilage tissue and muscle fibers. It is known that damage of cartilage and muscular tissue is a serious problem in, for example rheumatic diseases and sports injuries. The lack of blood supply

is one of the most important factors involved. Previous studies have proven the regeneration capacities of laser in wound healing and bone repair.

Material and Methods: In the first part of this study we have chosen to experiment on cartilage tissue of mice. A 0.4 mm hole was drilled in both ears on 15 mice. The mice were divided into 3 groups, dependent on the duration of treatment (1, 2 or 4 days). The right ears were treated with GaAs IR laser (904nm, 10W) for 3 minutes. The left ears remained untreated and were used as control. Photographs were taken from the histologic sections and were interpreted by using the Bentley method. Parameters were: macrophage activity, fibroblast proliferation, chondrocyte activity, remodeling of cartilage structure and perichondrium activity. The statistical analyses of the results was done by a Chi square test. The results were statistically analysed using Kolmogorov-Smirnov Goodness of fit test and Wilcoxon Matched-Pairs Singeld-Rank test. Significant level was always chosen at the 5% level.

Results: After 1 day of treatment, no significant differences were found between the irradiated group and control and there was no tendency of cell proliferation. After 2 days of treatment, the drilled hole is filled with cartilage structures and is more filled than the control ear. No activity of perichondrium and a differentiation of chondrocytes and fibroblasts is notable. A significant activation of the perichondrium is found towards the drilled hole. There is a significant difference between the irradiated and non irradiated side; the untreated ear is filled, but with totally other cell structures than in the treated ear.

2) Light Stimulated Hyaline Cartilage Formation

This study found that irradiation of articular cartilage lesions with infrared wavelengths (904 nm) resulted in formation of hyaline cartilage, while those irradiated with red wavelengths (633nm) formed fibrocartilage. Granulation tissue formed in the untreated joints.

Histological and Clinical Responses of Articular Cartilage to Low-level Laser Therapy: Experimental Study

I. Ruiz Calatrava, J.M.Santisteban Valenzuela, R.J.G0mez-Villamandos J.I.Redondo, J.C.G0mez-Villamandos, L.Aviga Jurado. Lasers in Medical Science 1997, 12:117-121 0268 - 892.

This study was carried out to evaluate the effects of low-level laser irradiation on experimental lesions of articular cartilage. A standard lesion was practised on the femoral trochlea of both hind limbs of 20 clinically normal Californian rabbits. These animals were divided into two groups of 10 individuals each, depending on the laser equipment used for treatment. One group was treated with HeNe laser (8 J cm², 632.8 nm wavelength) and the other with infra-red (IR) laser (8 J cm², 904 nm wavelength). In both groups, five points of irradiation to the right limb alone were irradiated per session for a total of 13 sessions, applied with an interval of 24 h between sessions. These points were the following: left and right femoral epicondyles, left and right tibial condyles and the centre of articulation. The distance between these points was approximately 1 cm. The untreated left limb was left as a control. During treatment, extension angle and periarticular thickness were considered. At the end of the treatment, samples were collected for histopathological study and stained with: Haematoxylin-Eosin, PAS and Done. The results show a statistically higher anti-inflammatory capacity of the IR laser (p<0.0001). The functional recovery was statistically similar for both treatments (p<0.176). Histological study showed, at the end of the treatment, hyaline cartilage in the IR group, fibrocartilage in the HeNe group and granulation tissue in the control limbs. Clinical and histological results indicated that this laser treatment had a clear anti-inflammatory effect that provided a fast recuperation and regeneration of the articular cartilage.

In this study a combination of red and blue wavelengths alone and in combination with magnetic fields. The light combinations alone stimulated hyaline repair while the addition of magnetic fields inhibited repair.

Effects of laser and magnetolaser treatment on the growth and maturation of repairing hyaline cartilage cells in the knee joint of rabbits in different postoperative periods.

Archakova L. I., Gourine V. N., Yemelyanova A. A., Serdyuchenko N. S., Soroka N. F.
Proceedings of the National Academy of Sciences of Belarus (Vestsi Natsiyanal'nai Akademii Navuk Belarusi) SERIES OF MEDICAL-BIOLOGICAL SCIENCES
(News of Biomedical Sciences) Published by The Belaruskaya Navuka Publishing House, Minsk, Republic of Belarus, Number 1, pp. 42—53; 2002.

Summary: On a model of traumatic arthritis of the knee joint in rabbits, effect of combined blue ($\lambda = 441.6$ nm) and red ($\lambda = 632.8$ nm) laser radiation and their effects in combination with low magnetic field of 25–50 mTl on the submicroscopic organization of a surgically damaged repairing hyaline cartilage at 21, 30, 60 and 90 days after operation in case of laser exposure and at 30 days in case of the magnetolaser treatment were studied using electron microscopy. During 21 postoperative days the animals received 14 sessions of combined laser radiation, and during 30, 60, and 90 days 18 sessions, respectively, like under magnetolaser treatment. The exposures either increased the proliferation of cartilage cells or facilitated their maturation and also led to degenerative changes of cartilage tissue which were more or less intense depending on experimental conditions. The most favorable effect on the repair of the hyaline cartilage was produced by 18 sessions of blue and red laser radiation 60 and 90 days after operation, when degenerative cartilage cells were detected only in single instances and the proliferation and maturation processes in chondrocytes were optimally balanced. At the same time, 18 sessions of combined treatment with magnetic field and blue and red laser radiation during 30 days appeared to be a potent stimulus which inhibited the repair processes in the injured cartilage, resulting in the death of many young chondrocytes after initial stimulation of the proliferation of cartilage cells.

This study demonstrates the effectiveness of light therapy on cartilage repair in an induced Osteoarthritis animal model. The appearance of the repaired cartilage in the treatment group suggests (though not specifically called out) that it is hyaline-like rather than fibrotic.

Effect of low-level laser therapy on osteoarthropathy in rabbit.

Cho HJ, Lim SC, Kim SG, Kim YS, Kang SS, Choi SH, Cho YS, Bae CS In Vivo. 2004 Sep-Oct;18(5):585-91.

The aim of this study was to determine whether low-level laser therapy (LLLT) aided the recovery of damaged articular cartilage in joints with artificially induced osteoarthropathy (OA). OA was induced by injecting hydrogen peroxide (H_2O_2) into the articular spaces of both knees in rabbits, twice a week for 4 weeks. The induction of OA and the effect of LLLT were evaluated by biochemical, radiological and histopathological analysis. Superoxide dismutase (SOD) activity increased about 40% in the OA group, as compared to the controls. Although SOD activity in the OA group was not significantly different from the 2-week groups, it was significantly different from the 4-week control and treatment groups. There was also a significant difference between the 4-week control and treatment groups. Simple radiographs and three-dimensional computed tomographs (3D CT) did not show detectable arthropathy in the OA group, nor any particular changes in the 2-week groups. In contrast, distinct erosions were seen in the distal articular cartilage of the femur, with irregularity of the articular surface, in the 4-week control group, while the erosions were reduced and arthropathy improved slightly in the 4-week treatment group. Grossly, erosions formed on the articular surface in the OA group. In comparison, severe erosions damaged the articular cartilage in the 4-week control group, but not in the 2-week control and treatment groups. Regeneration of articular cartilage was seen in gross observations in the 4-week treatment group. Histopathologically, there was slight irregularity of the articular surface and necrosis in the OA group, and serious cartilage damage, despite slight chondrocyte regeneration, in the 4-week control group. Conversely, the 4-week treatment group showed chondrocyte replacement, with sometimes close to normal articular cartilage on the articular surface. These results suggest that LLLT was effective in the treatment of chemically-induced OA.

3) Light Stimulated Synthesis of Extracellular Matrix Formation

Light therapy induces mucopolysaccharide, which plays a role in improved histopathological findings within arthritic cartilage.

Effects of helium-neon laser on the mucopolysaccharide induction in experimental osteoarthritic cartilage.

Lin YS, Huang MH, Chai CY. Osteoarthritis Cartilage. 2006 Apr;14(4):377-83. Epub 2005 Dec 13.

Objective: To investigate the effects of mucopolysaccharide induction after treatment by low power laser for experimental osteoarthritis (OA).

Methods: Seventy-two rats with three different degrees of papain induced OA over right knee joints were collected for helium-neon (He-Ne) laser treatment. The severity of induced arthritis was measured by 99mTc bone scan and classified into three groups (I-III) by their radioactivity ratios (right to left knee joints). The rats in each group were further divided into study subgroups (Is, IIs, and IIIs) and control subgroups (Ic, IIc, and IIIC) randomly. The arthritic knees in study subgroups received He-Ne laser treatment, and those in controls received sham laser treatment. The changes of arthritic severity after treatment and follow-up 2 months later were measured. The histopathological changes were evaluated through light microscope after disarticulation of sections (H.E. stain), and the changes of mucopolysaccharide density in cartilage matrix were measured by Optimas scanner analyzer after Alcian blue (AB) stain. The densities of mucopolysaccharide induced after treatment in arthritic cartilage were compared and correlated with their histopathological changes.

Results: The density of mucopolysaccharide rose at the initial stage of induced arthritis, and decreased progressively in later stages. The densities of mucopolysaccharide in treated rats increased upon complete laser treatment more than those of the controls, which is closely related with the improvement in histopathological findings, but conversely with the changes in arthritic severity.

Conclusion: He-Ne laser treatment will enhance the biosynthesis of arthritic cartilage, and results in the improvement of arthritic histopathological changes.

As noted above, chondrocytes of different types occupy different zones of cartilage and produce different components of extracellular matrix. This study focuses on articular chondrocytes and demonstrates light therapy stimulated growth and secretion of extracellular matrix. In this study, measurements of the products of mid-zone chondrocytes reveal biophotostimulation of these mid-zone chondrocytes.

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

Ya-Li Jia, Zhou-Yi Guo. Lasers Surg. Med. 34:323-328, 2004.

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/HamF12 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/cm². 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/cm² increased the cell numbers and revealed a considerably higher cell proliferation activity comparing to control cultures. The energy density of 4 and 5 J/cm² 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.

This study examines high energy vs. low energy laser treatment and suggests that, in contrast to high energy laser, low energy light treatment stimulates collagen production.

Control of connective tissue metabolism by lasers: recent developments and future prospects.

Abergel RP, Meeker CA, Lam TS, Dwyer RM, Lesavoy MA, Uitto J. J Am Acad Dermatol. 1984 Dec;11(6):1142-50.

Various laser modalities are currently in extensive use in dermatology and plastic surgery, particularly for treatment of vascular and pigmented lesions. A relatively new area of laser utilization involves the possible biologic effects of the lasers. In this overview, we are summarizing our recent studies, which indicate that lasers at specific wavelengths and energy densities modulate the connective tissue metabolism by skin fibroblasts both in vitro and in vivo. Specifically, the neodymium-yttrium-aluminum-garnet (Nd: YAG) laser was shown to selectively suppress collagen production both in fibroblast cultures and in normal skin in vivo, thus suggesting that this laser modality may be useful for the treatment of fibrotic conditions such as keloids and hypertrophic scars. Furthermore, two low-energy lasers, helium-neon (He-Ne) and gallium-arsenide (Ga-As), were shown to stimulate collagen production in human skin fibroblast cultures, suggesting that these lasers could be used for enhancement of wound healing processes. These experimental approaches illustrate the future possibilities for applying lasers for the modulation of various biologic functions of cells in tissues and attest to the potential role of lasers in the treatment of cutaneous disorders.

V. Other markers of cartilage healing in arthritis models as influenced by Light Therapies

Low level light therapy stimulates Stress Protein release, leading to improved cartilage healing. Stress Proteins appear when the cell is under stress. They also occur under non-stressful conditions, simply "monitoring" the cell's proteins. Some examples of their role as "monitors" are that they carry old proteins to the cell's "recycling bin" and they help newly synthesized proteins fold properly. These activities are part of a cell's own repair system, called the "cellular stress response." In this case, light-stimulated upregulation of stress proteins are an indicator of cartilage repair processes at work.

Effects of helium-neon laser on levels of stress protein and arthritic histopathology in experimental osteoarthritis.

Lin YS, Huang MH, Chai CY, Yang RC. American Journal of Physical Medicine & Rehabilitation. 83(10):758-765, October 2004.

Objective: To investigate the effect of low-power laser therapy on levels of stress proteins (SPs) in experimental arthritis and their relation to the bioeffects on arthritic cartilage repair.

Design: A total of 42 rats with similar degrees of induced arthritis evaluated by means of bone scan were divided randomly into two groups. In the treated group, 21 rats received helium-neon laser treatment; in the control group, 21 rats received sham laser treatment. The changes in chondrocytes of SPs were measured by electrophoresis of proteins extracted from chondrocytes of arthritic cartilage at various time periods. The histopathologic changes and the presence of SP of arthritic cartilage were identified by hematoxylin and eosin stain and by immunostains of SP72 antibody individually from frozen sections of arthritic cartilage.

Results: SP density increased markedly in rats after laser treatment and was closely related to the repair of arthritic cartilage. Furthermore, the pathohistology of arthritic cartilage improved significantly with the decline of SP levels in the follow-up period.

Conclusion: Helium-neon (632 nm) low-power laser can enhance SP production in arthritic chondrocytes. The extragenic production of SP is well correlated with the therapeutic effect of low-power laser in preserving chondrocytes and the repair of arthritic cartilage in rats.

Recent work points out evidence of an inflammatory component to the progression of OA.

Novel strategies for the treatment of osteoarthritis

Authors: Chikanza I.1; Fernandes L. Expert Opinion on Investigational Drugs, Volume 9, Number 7, July 2000, pp. 1499-1510(12)

Osteoarthritis is a worldwide heterogeneous group of conditions that leads to joint symptoms, which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins. The prevalence of the disease after the age of 65 years, is about 60% in men and 70% in women. The aetiology of osteoarthritis is multifactorial, with the end result being mechanical joint failure and varying degrees of loss of joint function. The pathophysiological events associated with osteoarthritis are beginning to be understood. Essential inflammatory cytokines, such as IL-1 and TNF- are involved initiating a vicious cycle of catabolic and degradative events in cartilage, mediated by metalloproteinases, which degrade cartilage extracellular matrix. The role of inflammation in the pathophysiology and progression of early osteoarthritis is supported further by the observation that C-reactive protein levels are raised in women with early knee osteoarthritis and higher levels predict those whose disease will progress. The synovium from osteoarthritis joints stains for IL-1 and TNF-. Nitric oxide, which exerts pro-inflammatory effects, is released during inflammation. Cartilage from patients with rheumatoid arthritis and osteoarthritis spontaneously produces nitric oxide in vitro. In experimental osteoarthritis, nitric oxide induces chondrocyte apoptosis, thus contributing to cartilage degradation. Hence unregulated nitric oxide production in humans plays a part in the pathophysiology of the disease. These recent observations suggest that therapy can now be targeted at specific sites of pathophysiological pathways involved in the pathogenesis of osteoarthritis. The novel strategies under consideration for the treatment of osteoarthritis can be divided into five main areas. These are COX-2 inhibitors, nitric oxide synthesis inhibitors and anti-oxidants, chondrocyte and bone growth promoters, metalloproteinase and cytokine inhibitors and gene therapy.

This study establishes a link between Light Therapy and Anti-inflammatory activity. Evidence show down-regulation of prostaglandin E2 at the transcriptional level.

Inhibitory effect of low-level laser irradiation on LPS-stimulated prostaglandin E2 production and cyclooxygenase-2 in human gingival fibroblasts.

Sakurai Y, Yamaguchi M, Abiko Y. *European Journal of Oral Science* 108: 29-34

It has been reported that lipopolysaccharide (LPS) from periodontal pathogens can penetrate gingival tissues and stimulate the production of prostaglandin E2 (PGE2), which is known as a potent stimulator of inflammation and bone resorption. Although biostimulatory effects of low-level laser irradiation such as anti-inflammatory results have been reported, the physiological

mechanism is not yet clarified. The purpose of the present study was to determine the effect of laser irradiation on PGE2 production and cyclooxygenase (COX)-1 and COX-2 gene expression in LPS-challenged human gingival fibroblast (hGF) cells in vitro. hGF cells were prepared from healthy gingival tissues and challenged with LPS, and Ga-Al-As diode laser was irradiated to the hGF cells. The amount of PGE2 released in the culture medium was measured by radioimmunoassay, and mRNA levels were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR). Irradiation with Ga-Al-As diode low-level laser significantly inhibited PGE2 production in a dose-dependent manner, which led to a reduction of COX-2 mRNA levels. In conclusion, low-level laser irradiation inhibited PGE2 by LPS in hGF cells through a reduction of COX-2 mRNA level. The findings suggest that low-level laser irradiation may be of therapeutic benefit against the aggravation of gingivitis and periodontitis by bacterial infection.

This study suggests a mechanism by which Light Therapy conveys and anti-inflammatory effect on the joint capsule. A host of pro-inflammatory factors are repressed.

Anti-inflammatory effect of linear polarized infrared irradiation on interleukin-1beta-induced chemokine production in MH7A rheumatoid synovial cells.

Shibata Y, Ogura N, Yamashiro K, Takashiba S, Kondoh T, Miyazawa K, Matsui M, Abiko Y. Lasers Med Sci.;20(3-4):109 Dec 13, 2005.

We examined the anti-inflammatory effect of infrared linear polarized light irradiation on the MH7A rheumatoid fibroblast-like synoviocytes (FLS) stimulated with the proinflammatory cytokine interleukin (IL)-1beta. Expression of messenger ribonucleic acids (mRNAs) encoding IL-8, RANTES (regulated upon activation, normal T cell expressed and secreted), growth-related gene alpha (GROalpha), and macrophage inflammatory protein-1alpha (MIP1alpha) was measured using real-time reverse transcription polymerase chain reaction, and the secreted proteins were measured in the conditioned media using enzyme-linked immunosorbent assays. We found that irradiation with linear polarized infrared light suppressed IL-1beta-induced expression of IL-8 mRNA and, correspondingly, the synthesis and release of IL-8 protein in MH7A cells. This anti-inflammatory effect was equivalent to that obtained with the glucocorticoid dexamethasone. Likewise, irradiation suppressed the IL-1beta-induced expression of RANTES and GROalpha mRNA. These results suggest that the irradiation of the areas around the articular surfaces of joints affected by rheumatoid arthritis (RA) using linear polarized light may represent a useful new approach to treatment.

In addition to downregulating prostaglandin E2, Light Therapy reduces levels of interleukin-1 beta (IL-1 β), a key regulator of cartilage degradation.

Inhibition of prostaglandin E2 and interleukin 1-beta production by low-power laser irradiation in stretched human periodontal ligament cells

N. Shimizu, M. Yamaguchi, T. Goseki, Y. Shibata, H. Takiguchi, T. Iwasawa and Y. Abiko. Journal of Dental Research, Vol 74, 1382-1388, 1995.

It is well-known that orthodontic treatment usually causes some discomfort and pain to the patients. Recently, it has been reported that low-power laser irradiation is effective in reducing the pain accompanying tooth movement. However, the mechanism of such pain relief cannot be elucidated. Since high levels of prostaglandin (PG) E2 and interleukin (IL)-1 beta are found in the periodontal ligament (PDL) during tooth movement, and both factors are involved in the induction of pain, the effects of low-power laser irradiation on PGE2 and IL-1 beta production in stretched human PDL cells were studied in vitro. The PDL cells, derived from healthy premolars extracted for orthodontic treatment, were utilized for experiments. Cells were seeded in flexible-bottomed culture plates, and the bottom of each plate was elongated (18% increase) under vacuum at 6 cycles per min for 1, 3, or 5 days. The stretched cells were irradiated with a Ga-Al-As low-power diode laser (60 mW) once a day for 3, 6, or 10 min (from 10.8 to 36.0 J) for 1, 3, or 5 days. PGE2 and IL-1 beta levels in the medium were measured by radioimmunoassay. In response to mechanical stretching, human PDL cells showed a marked elevation in PGE2 production in a

time-dependent manner. IL-1 beta production was also elevated, but this remained constant. The increase in PGE2 production was significantly inhibited by laser irradiation in a dose-dependent manner. The increase in IL-1 beta production was also significantly inhibited by laser irradiation, although the inhibition was only partial.

Finally, in a meta-analysis of Light Therapy clinical trials on OA, Bjordal, et al. noted that in trials where concomitant administration of non-steroidal anti-inflammatory drugs (NSAIDs) were allowed, the differences between treated and untreated patients was diminished compared to the differences between groups where no NSAIDs were allowed.

A systematic review of low level laser therapy with location-specific doses for pain from joint disorders.

Bjordal JM, Couppé C, Chow RT, Tunér J and Ljunggren AE (2003): Australian Journal of Physiotherapy 49: 107-116.

“The overall effect in trials that explicitly allowed anti-inflammatory drugs was poorer than those which did not allow for this co-intervention. This adds support for our hypothesis that pain reduction from LLLT is achieved through an anti-inflammatory action.” (pg. 113)

Light Therapy stimulates meniscal healing, as seen in the following two studies. As with other cartilage components and structures, progenitor cells are at the core of new cartilage formation. Meniscal cartilage consists of a preponderance of fibrocartilage. Differentiation of progenitor cells into Type I producing chondrocytes is a site-determined differentiation.

Effect of CO2 laser on healing of cultured meniscus

Tsai CL, Huang LL, Kao MC. Lasers Surg Med. 1997;20(2):172-8

Background and Objective: A new method to improve cartilage repair is clinically important. The enhancement of meniscal healing by low power CO2 laser was investigated in an organ culture system.

Study Design/Materials and Methods: A longitudinal or a radial defect was made in the avascular zone of rabbit menisci. Irradiation by CO2 laser with 1 W (energy density 50 J/cm²) and 2 W (energy density 100 J/cm²) was used.

Results: Histologic and scanning electron microscopic evaluations revealed that both energy densities of laser irradiation and the type of and the site of meniscal defect can influence the course and the outcome of meniscal healing. A marked increase in fibrochondrocytic proliferation and regeneration of collagen fibers were demonstrated in the meniscal defects irradiated by 100 J and CO2 laser energy.

Conclusions: The healing of meniscal defects could be promoted by low power CO2 laser irradiation.

The use of laser surgery in subtotal meniscectomy and the effect of low-level laser therapy on the healing potential of rabbit meniscus: an experimental study

N. Nicolopoulos, C. Cavadias, A. Karameris, M. Dyson, A. Tsourouktsoglou and P. Kondoyannis. Journal Lasers in Medical Science, Issue Volume 11, Number 2 Pages 109-115/ June, 1996.

The aim of this study was to evaluate the effect of low-level laser therapy on the healing potential of the peripheral rim of rabbit meniscus following laser subtotal meniscectomy, and to compare the subtotal meniscectomy as produced by a surgical blade and by a surgical CO2 laser. Twenty-four male New Zealand rabbits were divided into three groups (A, B and C). In each animal, a subtotal meniscectomy of the medial meniscus of the right knee was carried out. In Group A, meniscectomy was performed with a surgical blade, and in Groups B and C, a meniscectomy was performed with a CO2 laser (6 W, 0.1 mm, 0.2 s). The animals of Group C were treated every other day with a GaAlAs diode laser (820 nm, 100 mW, 48 J cm⁻²) until death. Two rabbits of

each group were killed on the 10th, 20th, 30th and 40th post-operative days. The medial meniscus was removed from all cases, fixed in buffered formalin and embedded in paraffin wax. Subsequently, the sections were stained with Haematoxylin and Eosin as well as with Masson's trichrome, and examined microscopically and morphometrically.

This study showed that: (1) fibroblasts and collagen fibrils were more abundant and dense in rabbits of Group C than in rabbits of Groups A and B; (2) after the 20th post-operative day, the granulation tissue was more cellular in Group C in comparison with Groups A and B; and (3) the morphometric evaluation showed that the subtotal meniscectomy with CO₂ laser in Groups B and C caused less tissue damage in comparison with the conventional surgical technique used in Group A. It was concluded that: (1) less damage was produced by a CO₂ laser than by a surgical blade; and (2) the healing potential of rabbit meniscus following laser resection was accelerated with the use of low-level laser therapy post-operatively.

VI. Human Studies of Cartilage Repair with Light Therapies

1) In Vitro Studies

Human studies tend to confirm findings in animal studies with regard to light stimulated chondrocyte formation as illustrated by the following study.

Biostimulation of human chondrocytes with Ga Al As diode laser: 'in vitro' research.

Morrone G., Guzzardella GA, Tigani D et al. Artificial Cells, Blood Substitutes, and Immobilization Biotechnology. 2000; 28 (2):193-201.

The aim of the study was to verify the effects of LLLT performed with GaAlAs (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 culture was divided into four groups. Groups I, II, III were subject to biostimulation with the following laser parameters:

- 300J, 1W, 100Hz, 10 min. exposure, pulsating emission
- 300J, 1W, 300Hz, 10 min. exposure, pulsating emission
- 300J, 1 W, 500Hz, 10 min. exposure, pulsating emission

Group IV did not receive any treatment. The laser biostimulation was conducted for five consecutive days. The data showed good results in terms of cell viability and levels of Ca and Alkaline Phosphate in the groups treated with laser compared to the untreated group. The results obtained confirm our previous positive in vitro results that the GaAlAs laser provide biostimulation without cell damage.

The synovium, the soft tissue which lines the non-cartilaginous surfaces within joints, is an active participant in cartilage health. In the following study the known link between synoviocytes and inflammatory processes is used to demonstrate anti-inflammatory effects of light. The study measures key pro-inflammatory markers at the transcriptional level to quantify the effects of light therapy.

Anti-inflammatory effect of linear polarized infrared irradiation on interleukin-1beta-induced chemokine production in MH7A rheumatoid synovial cells.

Shibata Y, Ogura N, Yamashiro K, Takashiba S, Kondoh T, Miyazawa K, Matsui M, Abiko Y. Lasers Med Sci.;20(3-4):109 Dec 13, 2005.

We examined the anti-inflammatory effect of infrared linear polarized light irradiation on the MH7A rheumatoid fibroblast-like synoviocytes (FLS) stimulated with the proinflammatory cytokine interleukin (IL)-1beta. Expression of messenger ribonucleic acids (mRNAs) encoding IL-8, RANTES (regulated upon activation, normal T cell expressed and secreted), growth-related gene

alpha (GROalpha), and macrophage inflammatory protein-1alpha (MIP1alpha) was measured using real-time reverse transcription polymerase chain reaction, and the secreted proteins were measured in the conditioned media using enzyme-linked immunosorbent assays. We found that irradiation with linear polarized infrared light suppressed IL-1beta-induced expression of IL-8 mRNA and, correspondingly, the synthesis and release of IL-8 protein in MH7A cells. This anti-inflammatory effect was equivalent to that obtained with the glucocorticoid dexamethasone. Likewise, irradiation suppressed the IL-1beta-induced expression of RANTES and GROalpha mRNA. These results suggest that the irradiation of the areas around the articular surfaces of joints affected by rheumatoid arthritis (RA) using linear polarized light may represent a useful new approach to treatment.

In this study infrared treatment on a short term basis, once daily for 12 days, demonstrated analgesic relief but no improvement in range of motion. Case study experience with LumiWave has shown much longer treatment periods (up to three months) to achieve significant improvement in range of motion and functionality. This makes sense, as cartilage regeneration is known to be a slow process compared to other body tissues. Short term studies showing improved range of motion or functionality are likely demonstrating the anti-inflammatory and swelling reduction effects of light treatment as opposed to cartilage regeneration. Longer term, intensive treatment protocols result in longer term relief and functionality improvements which may be linked to cartilage regeneration. (See meta-analysis further below.)

The Effect Of Low Power Laser Therapy On Osteoarthritis Of The Knee

Basirnia A., Sadeghipoor G., Esmaeeli Djavid G. et al. Lasers Florence 2003; <http://www.laser.nu>

Treatment was performed on 20 patients, aging from 42 to 60 years. All patients had received conservative treatment with poor results. Laser device used for this treatment was pulsed IR diode laser; 810 nm wavelength once per day for 5 consecutive days, followed by a 2-day interval. The total number of applications was 12 sessions. Irradiation was performed on 5 periarticular tender points, each for 2 min. The treatment outcome (pain relief and functional ability) was observed and measured according to the following methods: 1) Numerical rating scales (NRS), 2) Self assessment by the patient, 3) Index of severity for osteoarthritis of the knee (ISK), 4) Analgesic requirements. We achieved significant improvement in pain relief and quality of life in 70% of patients, comparing to their previous status ($p < 0.05$). There was no significant change in range of motion of the Knee.

In this study, not only were there differences between light therapy treated patients and untreated patients (showing significant improvement in treated over untreated in pain reduction and disability index improvement), but also between Infrared and Red wavelength treatments. The time from end-of-treatment until the patients required re-treatment was nearly 50% longer for infrared over red wavelengths, thus showing a greater long term effect from infrared.

Improvement Of Pain And Disability In Elderly Patients With Degenerative Osteoarthritis Of The Knee Treated With Narrow-Band Light Therapy (LLLT).

Jean Stelian, MD, Israel Gil, MD, Beni Habot, MD, Michal Rosenthal, MD, Iulian Abramovici, MD, Nathalia Kutok, MD, and Auni Khahil, MD. J Am Geriatr Soc. 1992; 40: 23-26.

Objective: To evaluate the effects of low-power light therapy (LLLT) on pain and disability in elderly patients with degenerative osteoarthritis in the knee.

Design: Partially double-blinded, fully randomized trial comparing red, infrared, and placebo light emitters.

Patients: Fifty patients with degenerative osteoarthritis of both knees were randomly assigned to three treatment groups: red (15 patients), infrared (18 patients) and placebo (17 patients). Infrared and placebo emitters were double-blinded.

Interventions: Self-applied treatment to both sides of the knee for 15 minutes twice a day for 10 days.

Main Outcome Measures: Short-Form McGill Pain Questionnaire, Present Pain Intensity, and Visual Analogue Scale for pain and Disability Index Questionnaire for disability were used. We evaluated pain and disability before and on the tenth day of therapy. The period from the end of the treatment until the patient's request to be retreated was summed up 1 year after the trial.

Results: Pain and disability before treatment did not show statistically significant differences between the three groups. Pain reduction in the red and infrared groups after the treatment was more than 50% in all scoring methods ($P < 0.05$). There was no significant pain improvement in the placebo group. We observed significant functional improvement in red and infrared treated groups ($p < 0.05$), but not in the placebo group. The period from the end of treatment until the patients required retreatment was longer for red and infrared groups than for the placebo group (4.2 ± 3.0 , 6.1 ± 3.2 , and 0.53 ± 0.62 months, for red, infrared, and placebo respectively)

Conclusions: Low-power light therapy is effective in relieving pain and disability in degenerative osteoarthritis of the knee. Degenerative osteoarthritis (DOA) is the most common rheumatic disorder of man and causes pain and disability especially in elderly people.

Following is a fairly large double-blind randomized and controlled trial showing statistically significant improvement in OA measures over placebo.

Efficacy of different therapy regimes of low-power laser in painful osteoarthritis of the knee: A double-blind and randomized-controlled trial

Gur Ali; Cosut Abdulkadir; Sarac Aysegul Jale; Cevik Remzi; Nas Kemal; Uyar Asur; Lasers in surgery and Medicine 2003, vol.33, no5, pp. 330-338.

Background and Objectives: A prospective, double-blind, randomized, and controlled trial was conducted in patients with knee osteoarthritis (OA) to evaluate the efficacy of infrared low-power Gallium-Arsenide (Ga-As) laser therapy (LPLT) and compared two different laser therapy regimes.

Study Design/Materials and Methods: Ninety patients were randomly assigned to three treatment groups by one of the nontreating authors by drawing 1 of 90 envelopes labeled:

'A' (Group I: actual LPLT consisted of 5 minutes, 3 J total dose + exercise; 30 patients),
'B' (Group II: actual LPLT consisted of 3 minutes, 2 J total dose + exercise; 30 patients),
'C' (Group III: placebo laser group+exercise; 30 patients).

All patients received a total of 10 treatments, and exercise therapy program was continued during study (14 weeks). Subjects, physician, and data analysts were unaware of the code for active or placebo laser until the data analysis was complete. All patients were evaluated with respect to pain, degree of active knee flexion, duration of morning stiffness, painless walking distance and duration, and the Western Ontario and Mc Master Universities Osteoarthritis Index (WOMAC) at week 0, 6, 10, and 14.

Results: Statistically significant improvements were indicated in respect to all parameters such as pain, function, and quality of life (QoL) measures in the post-therapy period compared to pre-therapy in both active laser groups ($P < 0.01$). Improvements in all parameters of the Group I and in parameters, such as pain and WOMAC of the Group II, were more statistically significant when compared with placebo laser group ($P < 0.05$).

Conclusions: Our study demonstrated that applications of LPLT in different dose and duration have not affected results and both therapy regimes were a safe and effective method in treatment of knee OA.

2) Meta-Analyses of Human clinical trials examining Light Therapy in OA

Following is the most comprehensive meta-analysis available in the literature on clinical effectiveness of light therapy in OA. The authors examined 88 randomized trials and



culled out studies based on subject (only chronic OA), design (blinded, appropriate and measurable outcomes) and dosage.

The authors conclude that, "The results of this review were surprisingly unequivocal in favor of active LLLT (low level light therapy) when dosage was titrated above the suggested lower dose limit for reduction of inflammation."

A systematic review of low level laser therapy with location-specific doses for pain from joint disorders.

Bjorndal JM, Couppé C, Chow RT, Tunér J and Ljunggren AE (2003): Australian Journal of Physiotherapy 49: 107-116.

We investigated if low level laser therapy (LLLT) of the joint capsule can reduce pain in chronic joint disorders. A literature search identified 88 randomised controlled trials, of which 20 trials included patients with chronic joint disorders. Six trials were excluded for not irradiating the joint capsule. Three trials used doses lower than a dose range nominated a priori for reducing inflammation in the joint capsule. These trials found no significant difference between active and placebo treatments. The remaining 11 trials including 565 patients were of acceptable methodological quality with an average PEDro score of 6.9 (range 5-9). In these trials, LLLT within the suggested dose range was administered to the knee, temporomandibular or zygapophyseal joints. The results showed a mean weighted difference in change of pain on VAS of 29.8 mm (95% CI, 18.9 to 40.7) in favour of the active LLLT groups. Global health status improved for more patients in the active LLLT groups (relative risk of 0.52; 95% CI 0.36 to 0.76). Low level laser therapy with the suggested dose range significantly reduces pain and improves health status in chronic joint disorders, but the heterogeneity in patient samples, treatment procedures and trial design calls for cautious interpretation of the results.

The following table summarizes the evaluable clinical studies' outcomes. The measures of pain are made on a 0 to 100 millimeter visual analogue scale (VAS). Of the 14 trials listed, 2 used sub-therapeutic doses, achieving non-significant results. Nine of the remaining 12 trials showed significance in favor of Light Therapy. Two of the three N.S. trials did not contain pain measures on VAS. The remaining trial shows treatment more effective than placebo but is underpowered.

Bjordal et al: A systematic review of low level laser therapy with location-specific doses for pain from joint disorders

Table 5. List of included trials with data on treatment outcome.

First author	No. of patients	Condition	Mean pain (mm) before treatment	Mean pain (mm) after treatment	Mean change in pain (mm)	Proportions of patients improved	Author's test of significance
<i>Basford 1987</i>	81	Active Placebo	53 48	(missing data) (missing data)		22/47 16/34	N.S.
<i>Jensen 1987</i>	29		No separate pain score (medication included)				N.S.
<i>Klein 1990</i>	20	Active Placebo	40 44	23 28	17 16		N.S.
<i>Stelian 1991</i>	50	Active(Red) Active(Infra) Placebo	65 72 62	33 32 63	32 40 -1		$p < 0.0001$ (Before/ after)
<i>Nivbrant 1992</i>	30	Active Placebo	67 58	44 54	23 4		$p < 0.01$ (Before/ after)
<i>Bulow 1994</i>	29	Active Placebo	82 71	61 69	21 2	7/14 9/15	N.S.
<i>Gray 1994</i>	55	Active Placebo				20/29 14/26	$p < 0.001$
<i>Toya 1994</i>	115	Active Placebo				43/59 16/56	$p < 0.0001$
<i>Bertolucci 1995</i>	32	Active Placebo				40 2	$p < 0.01$
<i>Gette 1995</i>	40	Active * Placebo*	69 70	42 68	27 2	13/20 2/20	"Significant" (no p-value)
<i>Conti 1997</i>	20	Active Placebo	58 49	27 38	31 11		N.S.
<i>Soriano 1998</i>	71	Active Placebo	79 81	(missing data) (missing data)		27/38 12/33	$p < 0.007$
<i>Basford 1999</i>	63	Active Placebo	35 37	17 33	18 4		$p < 0.001$
<i>Özdemir 2001</i>	60	Active Placebo	77 73	24 68	53 5		$p < 0.001$

Randomised placebo-controlled trials where LLLT has been used for CJD. Outcome data are extracted from trial reports. Trials shown in *italics* gave treatment outside suggested dose range. *Visual estimates of data from graphs in trial report. Blank boxes indicate missing data in trial report. N.S. = not statistically significant.

Six trials reported follow-up measurement of at least 3 weeks. Two trials reported pain reduction from light therapy for 4 – 6 months.

Short term studies showing improved range of motion or functionality are likely demonstrating the anti-inflammatory and swelling reduction effects of light treatment as opposed to cartilage regeneration. Longer term, intensive treatment protocols result in longer term relief and functionality improvements which may be linked to cartilage regeneration.

Duration of pain relief (Ibid)

Six trials with assumed optimal treatment employed follow-up measurement of at least three weeks. Four of these trials reported pain relief under blinded conditions (Basford et al 1999, Götte et al 1995, Gray et al 1994, Nivbrant and Friberg 1992). Two trials with intensive, daily treatment regimens (Soriano and Rios 1998, Stelian et al 1992) reported pain reduction from LLLT for four to six months, but evaluation in the followup period was unblinded.

The clinical goals in treating OA range from simple palliative pain relief, to slowing progression of cartilage destruction, to reversing cartilage destruction. The authors note that synovial inflammation worsens cartilage degeneration and that evidence points to an anti-inflammatory effect of light therapy in OA.

Anti-inflammatory effects of LLLT may slow or reverse cartilage degradation (Ibid).

A link has been established between synovial inflammatory activities and worsening of cartilage degeneration in osteoarthritis (Chikanza and Fernandes 2000). In this context, it is interesting to investigate if an anti-inflammatory action can be induced clinically by electrophysical agents.

Controlled laboratory trials have found that LLLT can reduce inflammation through reduction of PGE2-levels and inhibition of cyclooxygenase-2 (COX-2) in cell cultures (Campaña et al 1993, Honmura et al 1993, Sakurai et al 2000, Shimizu et al 1995).

The overall effect in trials that explicitly allowed anti-inflammatory drugs was poorer than those which did not allow for this co-intervention. This adds support for our hypothesis that pain reduction from LLLT is achieved through an anti-inflammatory action.

Dosage is a significant factor in evaluating trials. The authors present well reasoned criteria for appropriate dosage based on in vitro and in-vivo data. Many studies under dose based on measures of tissue penetration of differing wavelengths of light and demonstrated biological activity response.

Determination of possible anti-inflammatory LLLT dose at target location (Ibid).

In in vitro trials, LLLT has been reported to suppress inflammation by a reduction of PGE2 in ligament cell cultures (Sakurai et al 2000, Sattayut et al 1999, Shimizu et al 1995). Low level laser therapy has also been found to reduce PGE2 levels in the joint capsule of animals in in vivo trials (Campaña et al 1993 and 1999, Honmura et al 1993, Sakurai et al 2000). This effect was reported within a range between 0.4 and 19 J and a power density of 5-21.2 mW/cm². The lower range limits for PGE2 reduction were identified because data showed no effect below this threshold. Upper range limits could not be identified, as there were no data available to show when or if this effect would level off. However, it has been shown that power densities above 20 mW/cm² temporarily inhibit fibroblast metabolism (van Breugel and Bar 1992), and numerous fibroblast cells are found in the joint capsule. We assumed doses of 0.4-19 J and power density of 5-21 mW/cm² would be capable of reducing inflammation at the target joint capsule without compromising fibroblast metabolism.

VII. Clinical Applications of Light Therapy for rebuilding Cartilage in OA

1) Light stimulation of cartilage surface-dwelling progenitor cells

Foundational to rebuilding damaged cartilage is the activation, proliferation and differentiation of progenitor cells, specifically mesenchymal stem cells (MSC). In normal cartilage, chondrocytes provide house-keeping functions in maintaining the health and integrity of cartilage. Trauma and age-related break-down of repair processes, accompanied by resultant inflammatory responses within the joint lead to a situation where the repair processes are overwhelmed. This leads to a downward spiral of tissue damage and inflammatory cycles leading to greater damage.

If caught early enough, OA may be treated with infrared light therapy. The proposed mechanism of action is through stimulation of surface progenitor cells.

The surface of articular cartilage contains a progenitor cell population

Gary P. Dowthwaite, Joanna C. Bishop, Samantha N. Redman, Ilyas M. Khan, Paul Rooney, Darrell J. R. Evans, Laura Houghton, Zubeyde Bayram, Sam Boyer, Brian Thomson, Michael S. Wolfe and Charles W. Archer. Journal of Cell Science 117, 889-897 (2004)

Using differential adhesion to serum fibronectin, we have described the isolation and partial characterisation of a subpopulation of articular cartilage chondrocytes with properties akin to those of a progenitor cell and that are able to engraft into a variety of tissue types, albeit of the connective tissue lineage. These cells reside within the surface zone of articular cartilage, where the EDA isoform of fibronectin is differentially expressed and the cells have an extended cell cycle time (Hayes et al., 2001).

The existence of a progenitor population within the surface zone of articular cartilage opens up the possibility of using this population to engineer cartilage in vitro. Because these cells are undifferentiated, they should have the capability to reproduce the structural and hence biomechanical properties of normal articular cartilage and thus integrate more fully into articular cartilage lesions.

2) Microfracture Technique

A technique pioneered by Steadman for building cartilage in-vivo and which has gained popularity in recent years, is “microfracture,” described below. The technique involves bringing mesenchymal stem cells, growth factors and other cartilage-building proteins to the surface of the cartilage where they differentiate into hyaline cartilage, filling in the surface defects. These are the same cells and factors that are stimulated by infrared light. This suggests a potentially synergistic therapeutic combination of microfracture and light therapy to repair OA damaged cartilage.

Microfracture: Surgical Technique and Rehabilitation to Treat Chondral Defects.

Association Of Bone And Joint Surgeons Workshop Supplement.

Steadman, J. Richard MD; Rodkey, William G. DVM; Rodrigo, Juan J. MD. Clinical Orthopaedics & Related Research. 391 Supplement:S362-S369, October 2001.

Full-thickness articular cartilage defects rarely heal spontaneously. Some patients may not have clinically significant problems from chondral defects, but most eventually have degenerative changes. Techniques to treat chondral defects include abrasion, drilling, autografts, allografts, and cell transplantation. The senior author (JRS) developed the microfracture technique to enhance chondral resurfacing by providing a suitable environment for new tissue formation and taking advantage of the body's own healing potential. Microfracture has been done in more than 1800 patients. Specially designed awls are used to make multiple perforations, or microfractures, into the subchondral bone plate. Perforations are made as close together as possible, but not so close that one breaks into another. They usually are approximately 3 to 4 mm apart. The integrity of the subchondral bone plate must be maintained. The released marrow elements (including mesenchymal stem cells, growth factors, and other healing proteins) form a surgically induced super clot that provides an enriched environment for new tissue formation. The rehabilitation program is crucial to optimize the results of the surgery. It promotes the ideal physical environment for the marrow mesenchymal stem cells to differentiate into articular cartilage-like cells, ultimately leading to development of a durable repair cartilage that fills the original defect.

High-Impact Athletics After Knee Articular Cartilage Repair: A Prospective Evaluation of the Microfracture Technique

Kai Mithoefer, MD*, Riley J. Williams, III, MD., Russell F. Warren, MD, Thomas L. Wickiewicz, MD and Robert G. Marx, MD. The American Journal of Sports Medicine 34:1413-1418 (2006)

Background: Knee articular cartilage injuries in athletes present a therapeutic challenge and have been identified as an important cause of permanent disability because of the high mechanical joint stresses in athletes.

Purpose: To determine whether microfracture treatment of knee articular cartilage injuries can return athletes to high-impact sports and to identify the factors that affect the ability to return to athletic activity.

Study Design: Case series; Level of evidence, 4.

Methods: Thirty-two athletes who regularly participated in high-impact, pivoting sports before articular cartilage injury were treated with microfracture for single articular cartilage lesions of the knee. Functional outcome was prospectively evaluated with a minimum 2-year follow-up by

subjective rating, activity-based outcome scores, and the ability for postoperative participation in high-impact, pivoting sports.

Results: At last follow-up, 66% of athletes reported good or excellent results. Activity of daily living, Marx activity rating scale, and Tegner activity scores increased significantly after microfracture. After an initial improvement, score decreases were observed in 47% of athletes. Forty-four percent of athletes were able to regularly participate in high-impact, pivoting sports, 57% of these at the preoperative level. Return to high-impact sports was significantly higher in athletes with age <40 years, lesion size <200 mm², preoperative symptoms <12 months, and no prior surgical intervention.

Conclusion: Microfracture is an effective first-line treatment to return young athletes with short symptomatic intervals and small articular cartilage lesions of the knee back to high-impact athletics.

3) Tissue Transplantation

Low level laser has been shown to stimulate differentiation and proliferation of mesenchymal cells on a biomatrix for implantation. This review looks at the application to cartilage regeneration.

Directing Stem Cell Differentiation into the Chondrogenic Lineage In Vitro

Boon Chin Heng, Tong Cao, Eng Hin Lee. Stem Cells 2004;22:1152-1167 www.StemCells.com

A major area in regenerative medicine is the application of stem cells in cartilage tissue engineering and reconstructive surgery. This requires well-defined and efficient protocols for directing the differentiation of stem cells into the chondrogenic lineage, followed by their selective purification and proliferation in vitro. The development of such protocols would reduce the likelihood of spontaneous differentiation of stem cells into divergent lineages upon transplantation, as well as reduce the risk of teratoma formation in the case of embryonic stem cells. Additionally, such protocols could provide useful in vitro models for studying chondrogenesis and cartilaginous tissue biology. The development of pharmacokinetic and cytotoxicity/genotoxicity screening tests for cartilage-related biomaterials and drugs could also utilize protocols developed for the chondrogenic differentiation of stem cells. Hence, this review critically examines the various strategies that could be used to direct the differentiation of stem cells into the chondrogenic lineage in vitro.

To further optimize the culture milieu for chondrogenic differentiation, it may be worthwhile to look at other biophysical parameters in addition to the culture media composition. This would include varying oxygen tension [196, 197] and temperature within the incubator, as well as the application of physical stimuli in the form of electrical [198] and electromagnetic [199] fields, mechanical forces [200], heat shock [201], ultrasound [202], and even laser irradiation [203]. Indeed, evidence from the scientific literature suggests that the pathway of chondrogenic differentiation can be profoundly influenced by variations in such biophysical parameter.

The study below looks at MSCs that differentiate into bone forming cells. The same principles apply to MSC in cartilage matrix which differentiate into chondrocytes.

Low level laser irradiation stimulates osteogenic phenotype of mesenchymal stem cells seeded on a three-dimensional biomatrix

Liat Abramovitch-Gottlib, Talia Gross, Doron Naveh, Shimona Geresh, Salman Rosenwaks, Ilana Bar, and Razi Vago. Lasers in Medical Science Issue Volume 20, Numbers 3-4 / December, 2005

Mesenchymal stem cells (MSCs) seeded on three-dimensional (3D) coralline (*Porites lutea*) biomatrices were irradiated with low-level laser irradiation (LLLI). The consequent phenotype modulation and development of MSCs towards ossified tissue was studied in this combined 3D biomatrix/LLLI system and in a control group, which was similarly grown, but was not treated by

LLLI. The irradiated and non irradiated MSC were tested at 1–7, 10, 14, 21, 28 days of culturing via analysis of cellular distribution on matrices (trypan blue), calcium incorporation to newly formed tissue (alizarin red), bone nodule formation (von Kossa), fat aggregates formation (oil red O), alkaline phosphatase (ALP) activity, scanning electron microscopy (SEM) and electron dispersive spectrometry (EDS). The results obtained from the irradiated samples showed enhanced tissue formation, appearance of phosphorous peaks and calcium and phosphate incorporation to newly formed tissue. Moreover, in irradiated samples ALP activity was significantly enhanced in early stages and notably reduced in late stages of culturing. These findings of cell and tissue parameters up to 28 days of culture revealed higher ossification levels in irradiated samples compared with the control group. We suggest that both the surface properties of the 3D crystalline biomatrices and the LLLI have biostimulatory effects on the conversion of MSCs into bone-forming cells and on the induction of ex-vivo ossification.

¹ Inducible nitric oxide synthase in human diseases. K-D Kröncke, K Fehsel, and V Kolb-Bachofen. Clin Exp Immunol. 1998 August; 113(2): 147–156.

² Down Regulation of Renal Endothelial Nitric Oxide Synthase Expression in Experimental Glomerular Thrombotic Microangiopathy. Xin J Zhou, et al. Lab Invest 2000, 80:1079–1087.

³ The Photobiological Effect of Low Level Laser Radiation Therapy. Laser Therapy, Vol. 3, No. 1, Jan - Mar 1991.

⁴ Low-Energy Laser Therapy: Controversies and New Research Findings. Jeffrey R. Basford, M.D. Lasers in Surgery and Medicine 9:1-5, Mayo Clinic, Rochester, Minnesota, 1989

⁵ A systematic review of low level laser therapy with location-specific doses for pain from joint disorders. Bjordal JM, Couppé C, Chow RT, Tunér J and Ljunggren AE (2003): Australian Journal of Physiotherapy 49: 107-116.