Treatment of human cartilage defects by means of Nd:YAG Laser Therapy

Article *in* Journal of Biological Regulators and Homeostatic Agents · October 2012

Source: PubMed

8 authors, including:

Alessandro Zati
University of Bologna
37 PUBLICATIONS **312** CITATIONS
SEE PROFILE

Giovanna Desando
Istituto Ortopedico Rizzoli
52 PUBLICATIONS **1,322** CITATIONS
SEE PROFILE

Carola Cavallo
Istituto Ortopedico Rizzoli
67 PUBLICATIONS **2,074** CITATIONS
SEE PROFILE

Roberto Buda
Istituto Ortopedico Rizzoli
153 PUBLICATIONS **4,687** CITATIONS
SEE PROFILE

JOURNAL OF BIOLOGICAL REGULATORS & HOMEOSTATIC AGENTS Vol. 26, no. 4, 701-711 (2012)

TREATMENT OF HUMAN CARTILAGE DEFECTS BY MEANS OF Nd:YAG LASER THERAPY

A. ZATI[1], G. DESANDO[2], C. CAVALLO[3], R. BUDA[4], S. GIANNINI[4], D. FORTUNA[5], A. FACCHINI[2,3,6] and B. GRIGOLO[2,3]

[1]SC Department of Rehabilitation and Functional Recovery; [2]SC Laboratory of Immunorheumatology and Tissue Regeneration; [3]SSD Laboratory RAMSES; [4]SC II Orthopaedic and Traumatology Clinic, Rizzoli Orthopaedic Institute; Bologna, Italy; [5]Photobiolab (El. En. S.p.A.) Calenzano Firenze Italy; [6]Department of Internal Medicine and Gastroenterology; University of Bologna; Bologna, Italy

Received December 7, 2011 – Accepted June 6, 2012

Articular cartilage lesions represent a challenging problem for orthopaedic surgeons. The purpose of this study was to evaluate the effect of a new pulsed Nd:YAG High Intensity Laser Therapy on the regeneration of cartilage tissue in patients with traumatic lesions. Clinical, histological and immunohistochemical evaluations were performed. Ten patients affected by chondral lesions scheduled for ACI procedure, were enrolled into the study. During the chondrocyte expansion for ACI procedure, cartilage from five patients was treated by Nd:YAG High Intensity Laser Therapy (HILT group). No laser treatment was performed in the remaining patients, who were used as controls. Cartilage repair was assessed by clinicians using two different scores: Cartilage Repair Assessment (CRA) and Overall Repair Assessment (ORA). Cartilage biopsy specimens were harvested to perform histological and immunohistochemical analyses at T0 (before laser treatment) and T1 (at the end of the treatment). A significant decrease in cartilage depth was noticed in the HILT group at T1. Histological and immunohistochemical evaluations showed some regenerative processes in cartilaginous tissue in terms of high amount of proteoglycans, integration with adjacent articular cartilage and good cellular arrangement in the HILT group. By contrast, a not well organized cartilaginous tissue with various fibrous features in the control group at T0 and T1 was observed. In conclusion, the use of this new pulsed Nd:YAG HILT resulted promising in the treatment of moderate cartilage lesions markedly in the young patients.

An important problem in orthopedics is represented by cartilage lesions which heal incompletely or without full structural integrity (1, 2). Articular cartilage has a very limited intrinsic healing potential, due to the absence of vascularization and the presence of few and very specialized cells with low mitotic activity (3). Cartilage repair requires a multistep controlled activation and silencing of genes producing extracellular matrix, growth factors, adhesion molecules involved in tissue formation (4). Therefore, injury to the cartilage tissue represents the major challenge for orthopaedic

Key words: nd-yag laser, cartilage, chondral defects, histology

Mailing address: Dr Brunella Grigolo
Laboratory of Immunorheumatology
and Tissue Regeneration, Laboratory RAMSES,
Rizzoli Orthopaedic Institute,
Via di Barbiano 1/10, 40136 Bologna, Italy
Tel.: +39 051 6366803 Fax: +39 051 6366807
e-mail: brunella.grigolo@ior.it

0393-974X (2012)

surgeons. Historically, attempts to regenerate, heal, or stimulate the metabolism of damaged or senescent cartilage tissue included several procedures such as debridement (5), drilling of subchondral bone (6), microfractures (7), periosteal and perichondral transplantation (8) and Autologous Chondrocyte Implantation (ACI) (9, 10). Recently, the use of bone marrow derived cells has been employed in order to regenerate damaged cartilage (11, 12).

Although these therapies lead to a considerable improvement in the regeneration of cartilage tissue, they represent invasive procedures which needed to be performed in operatory room. In the early 1990s, Low Level Laser Therapy (LLLT) became a popular strategy for treating joint diseases such as rheumatoid arthritis and musculoskeletal pain (13,14) but its application for cartilage repair showed poor results. The effects of low dose of Neodymium: Yttrium Aluminum Garnet (Nd:YAG) laser therapy were evaluated by Hardie et al. for the treatment of full-thickness cartilage defects in an animal model. However, no sign of cartilage healing in the laser treated group was observed (15).

Further studies on the effects exerted by non contact low level continuous-wave Nd:YAG laser beam were performed by Spivak et al. who demonstrated in *vitro* the onset of an early process of cartilage regeneration (16).

Interesting results for the care of some musculoskeletal disorders were observed by using the High Intensity Laser Therapy (HILT), successfully employed to manage pain thanks to its anti-inflammatory and analgesic effects (17-20). Few studies have been performed to investigate the effects of this treatment on cartilage repair at histological and cellular levels but none of them is reported in Science Citation Report journals (21).

Some *in vivo* analyses performed by Schultz et al. demonstrated as a continuous high power Nd:YAG laser treatment is a valid tool in stimulating the cartilage regeneration in guinea pigs (22). These data were confirmed by preclinical studies which were performed on an osteoarthritis chicken model (23).

In the light of these findings, the aim of the current study was to determine whether a pulsed HILT is able to heal cartilaginous lesions in human knee joints. To this end, patients with chondral defects scheduled for ACI were enrolled in the study. Clinical, histological and immunohistochemical investigations on the cartilaginous tissue were carried out before (T0) and after laser treatment (T1). These preliminary findings gave evidence on the formation of new hyaline tissues in the treated group compared to the controlled one, showing that high intensity laser is probably necessary to prompt a consistent and clinically effective cartilage tissue regeneration.

MATERIALS AND METHODS

Patient data

A pilot study was designed and started with patients scheduled for two-step ACI surgery for the treatment of cartilage lesions in the knee joint. Ten patients affected by chondral lesions entered into the study (eight men and two women, mean age 33, range 17-50 years). In particular, five patients received HILT therapy (HILT group). No laser treatment was performed in the remaining patients who were used as controls. Lesions were arthroscopically debrided at time 0 (T0) during the first step of ACI treatment, necessary to harvest cartilage tissue for chondrocyte isolation. A second arthroscopic look was performed upon 45 to 60 days from time T0 (T1), at the time of chondrocyte implantation. Cartilage biopsy specimens were harvested at T0 and T1. Informed consent was obtained from all the patients who were enrolled into the study, and the work was approved by the Ethics Committee of Istituto Ortopedico Rizzoli (IOR). Patients did not receive any chronic pharmacological therapy but reported occasional use of ketoprofen 50 mg/die.

Lesion data (Lesion size assessment: ICRS Classification System)

The International Cartilage Repair Society (ICRS) classification system was used to assess cartilage lesions. This system is based on two criteria: the depth of cartilage lesions (0-4) and the area of cartilage damage (graded from normal to severely abnormal using the IKDC system) (28). The patients investigated in this study showed a single lesion in the knee joint with a mean size of 2.4 cm^2. The area of the cartilage lesions was calculated by a new software (Antology, EIEn, Calenzano, Firenze, Italy) which permits an analogical analysis reported in pixels (Fig. 1).

Laser irradiation

No laser irradiation was performed in the control group; while the specimens from HILT- group were irradiated with a high power pulsed laser (El.En. Calenzano, Firenze, Italy). The features of laser treatment are reported in detail in Table I. HILT treatment was

performed transcutaneously by a scan modality throughout four optical windows. Fifteen laser treatments were performed during three weeks. Laser intensity and dose parameters were chosen on the basis of some preliminary findings on a sheep animal model (data not shown).

Cartilage repair and overall repair assessments

Cartilage repair was evaluated using a system based on three parameters: the cartilage thickness, the integration of the repair tissue with adjacent one and the macroscopic appearance of cartilage surface. In more detail, the cartilage repair was assessed by two different scores: Cartilage Repair Assessments (CRA) reporting a range from 1 (severely abnormal cartilage to 12 (normal cartilage) and Overall Repair Assessments (ORA) score with a grading system from I (normal cartilage) to IV (severely abnormal cartilage) (24).

Histological analysis

Histological and immunohistochemical analyses were carried out on arthroscopic biopsies at T0 and T1 respectively. Samples were firstly fixed in 10% buffered formalin, then washed and decalcified with a 4% (v/v) HCl and 5% (v/v) formic acid solution at Room Temperature (RT) for about five days. The samples were then, dehydrated through a graded series of alcohol and embedded in paraffin. Five micrometer thick sections were cut from the paraffin block, and coated onto glass slides. Staining with 0.1 Safranin-O (Sigma) and 0.02% Fast Green (Sigma) diluted respectively in aqueous solution were performed for proteoglycan assessment.

The impact of laser treatment in cartilage lesions was estimated by using a modified cartilage scoring system by Pineda et al. and Wakitani et al. (25-26). The choice of the following histological scoring system had the purpose to render the samples more comparable between them, giving the presence of fragments of tissue from patients lacking orienting landmarks. This scoring system takes into account five parameters: cell morphology (0-4), matrix staining (0-3), surface regularity (0-3), thickness of cartilage (0-2) and integration of the new cartilage with the host cartilage (0-2); it has a maximum score of 14 points and a minimum of 0, that translates to a completely healthy cartilage. The evaluations were performed by three different investigators who were "blinded" to which group the patients were in.

Immunohistochemical analysis

For immunohistochemical analyses, specimens were deparaffinized and treated respectively with Mouse anti-human collagen type I Monoclonal antibody (Chemicon International, Temecula, CA, USA) and Mouse anti human collagen type II Monoclonal antibody (Chemicon International). Epitope unmaskings were performed by a treatment with 0.1% hyaluronidase (Sigma) in Phosphate Buffered Saline (PBS) at 37°C for 5 minutes. After washes, the slides were incubated with 0.04 M Trizma Base Saline (TBS) containing 0.1% Bovine Serum Albumin (BSA) (Sigma) for 5 mins at RT to prevent non-specific bindings. The slides were incubated with 2 µg/ml anti-collagen type I and 5 µg/ml anti-collagen type II diluted in 0.04 M TBS containing 1% and 0.1 % Triton X-100 for 1 h at RT. The slides were washed three times with 0.04 M TBS and incubated with biotinylated secondary Antibodies (BioGenex, San Ramon, CA, USA) for 20 minutes at RT. After three washes with 0.04M TBS the samples were incubated with an enzyme-labelled streptavidin (Biogenex) for 20 mins at room temperature, and after washes the reactions were developed using fast red substrate (Biogenex). Negative controls were performed by omitting the primary antibody. Slides were counterstained with haematoxylin and mounted in glycerol gel. All the samples were analyzed using a Microscope Eclipse 90i (Nikon).

Statistical analysis

Statistical analyses were carried out with Instat software (GraphPad, California). Unless otherwise stated, all analyses were made with the Mann Whitney non parametric test, which is proper for studies with small numbers of replicates. A *t*-test with the Welch correction was also performed.

RESULTS

All patients who received HILT treatment did not report any clinical adverse events. In detail, no skin burning, no edema, no inflammation were observed.

Lesion data

Patients treated by HILT showed in general a good regeneration of the cartilaginous tissue. The depth of cartilage lesions was significantly reduced in the HILT group whether compared to the control one ($P<0.01$). The majority of patients showed deep cartilage defects at T0. In the HILT group, a reduction in the depth of cartilage lesions was observed at T1. In particular, cartilage thickness was normal in one case (grade 0) as well evident by arthroscopic analysis (Fig. 2B), some superficial lesions were observed in two cases (grade 1) and some defects which involved less than 50% of the cartilage thickness were reported in two cases (grade 2). By contrast, an extension of lesion depth into the

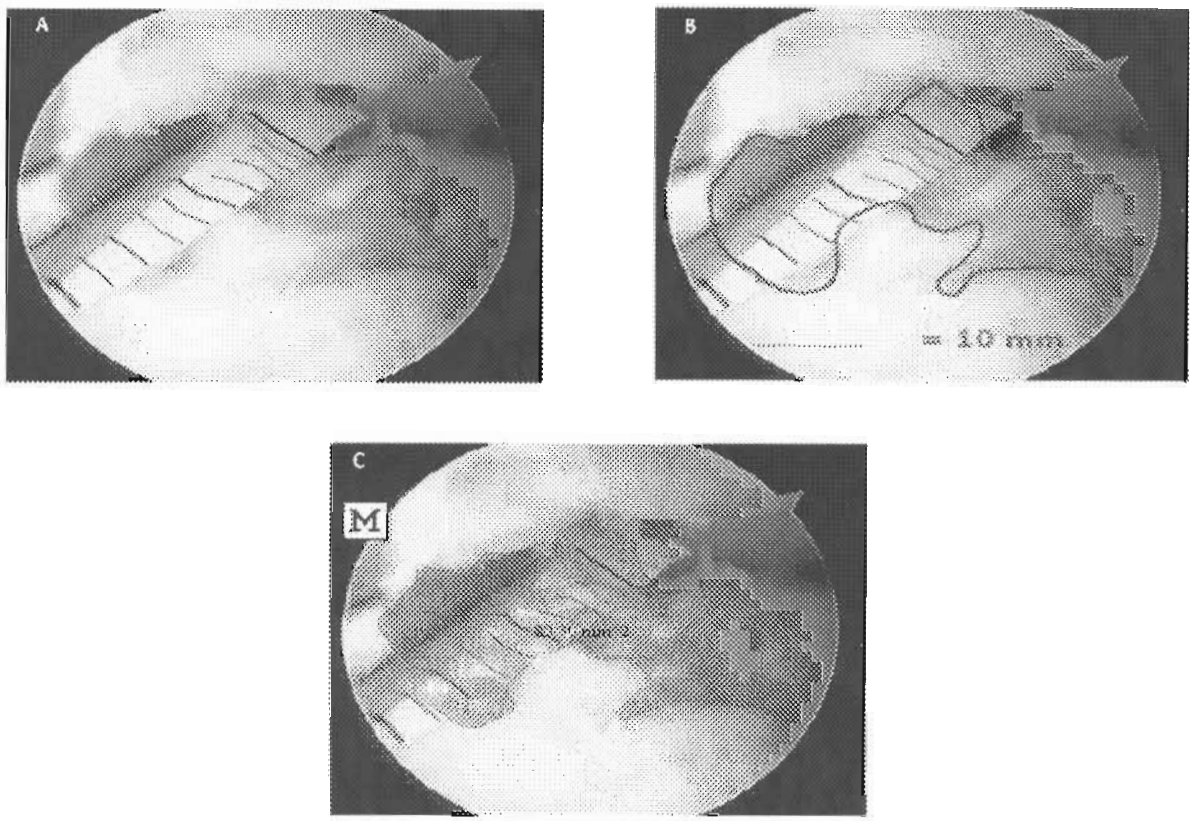

Fig. 1. *Calculation of the lesion area of one specimen by a new software which permits an analogical analysis in pixels (Anthology, DEKA, Firenze, Italy).*

subchondral bone (grade 3-4) was observed in all the patients from control group (Fig. 2D).

The cartilage lesions showed an area of approximately 4.0 cm^2 in the HILT group and 3.8 cm^2 in the controlone at T0. At T1, the cartilage lesions were completely covered in the HILT group whereas no filling processes were detected in the control one ($P<0.01$) with the exception of one case, where a reduction of lesion size was found. All the objective and subjective scores performed on cartilage tissue before and after laser treatment are reported in Table II.

Cartilage repair and overall repair assessments

The CRA score was significantly increased in the HILT group compared to the control one (7.0±3.3 versus 1.4±1.6 points) ($P<0.01$). Regardless to ORA assessment, a grade from I to III was observed in the HILT group and a grade IV in the control one.

Histological analysis

Histological analysis performed by Safranin-O/Fast Green staining in the control group, showed the presence of a not well organized cartilaginous tissue with various fibrous features at T0 (Fig. 3A). No signs of spontaneous repair processes were detected at T1 (Fig. 3B). HILT group showed a fibrous cartilaginous tissue at T0 (Fig. 3A). By contrast, some processes of cartilage repair in terms of a regular cellular arrangement, an integration with the surrounding tissue and a high GAG amount in four of five patients treated with laser therapy were evident at T1 (Fig. 3D). The semi-quantitative histological score showed a value significantly lower for HILT group compared to the control one ($P=0.031$) as indicated in table III (Fig. 4). Moreover, a significant

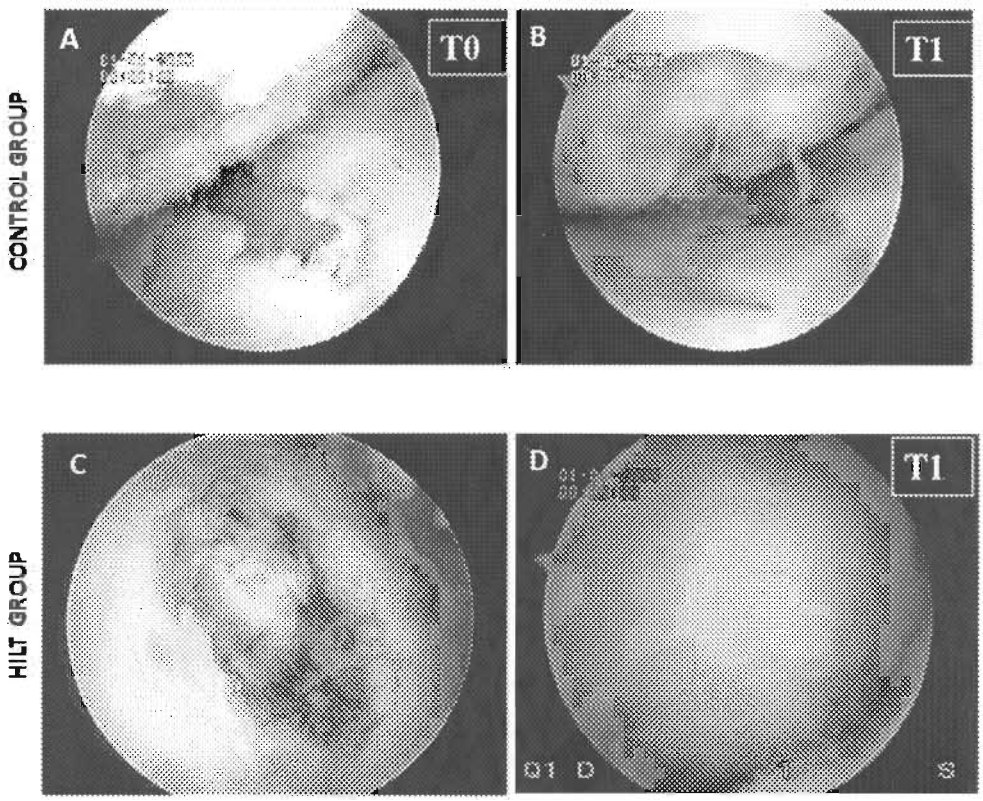

Fig. 2. *Arthroscopic analysis of control and HILT groups at T0 and T1. In the control group, at T0 cartilage lesion is well evident (A), no repair processes are observed at T1 (**B**). In the treated group the cartilage lesion is evident at T0 (C) while a good regeneration of the surface is observed at T1 (**D**).*

correlation between age and histological score (r = 0.97; p = 0.017) was observed in the HILT group.

Immunohistochemical analysis

A slight pattern of expression for type II collagen was detected at T0 in the control group (Fig. 5A). An intense positivity was instead observed in the superficial layer at T1 (Fig. 5A, B).

The cartilage from HILT group displayed some areas markedly positive for type II collagen at T0 (Fig. 5C). A strong expression level for type II collagen along all the thickness of specimens was noticed at T1 (Fig. 5D).

Immunostaining for type I collagen showed an intense positivity at cellular level in the control group at both T0 and T1 (Fig. 6, A, B). Intense positive staining for type I collagen was also found in the HILT group at T0. By contrast, a slight expression was detected for laser group at T1 (Fig. 6 C, D).

DISCUSSION

Different surgical techniques are employed for the repair of damaged articular cartilage in clinics. All these procedures tend to restore the morphological and functional properties of the cartilaginous tissue. Despite some treatments, particularly cell-based approaches, provide good results in the regeneration of articular cartilage, they often require one or more surgical interventions (5-12).

Laser therapy is gaining popularity in a variety of clinical applications such as ophthalmology (27) dermatology (28) and dentistry (29) showing an important role in reducing pain (30) and controlling inflammation (31). Several studies reported many positive physiological effects by laser treatment on

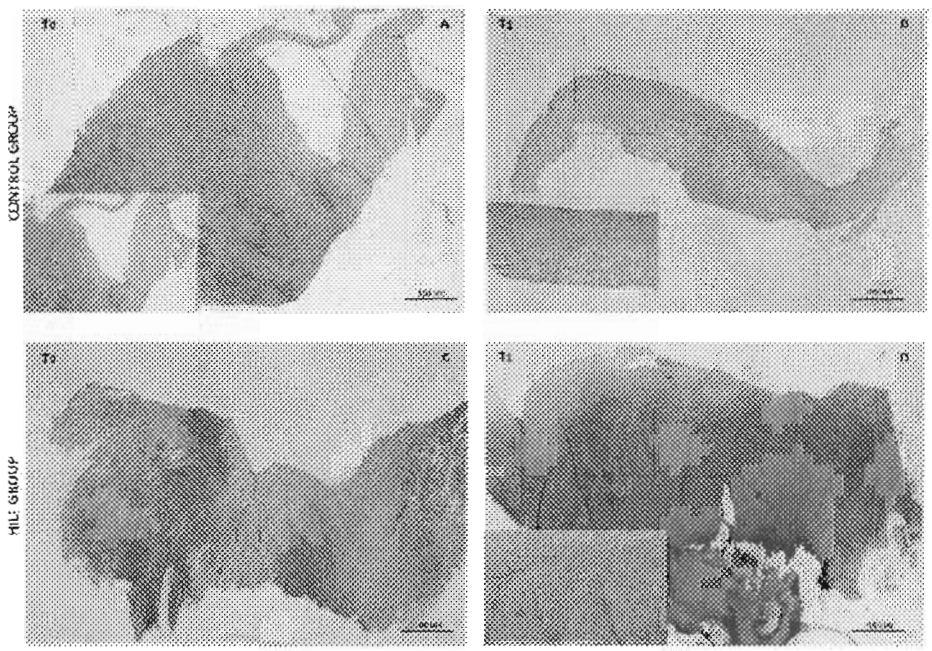

Fig. 3. *Histological analyses of control and HILT groups at T0 and T1. Sections are stained with Safranin-O to assess proteoglycan content in articular cartilage matrix and Fast Green to assess bone and fibrous tissue presence. Sagittal sections of a representative case of control group at T0 (**A**) and T1 (**B**). In this group is well evident the presence of a fibrous tissue, low proteoglycan component at T0; a worst scenario with high collagen content is observed at T1. Sagittal sections of a representative case of HILT group at T0 (**C**) and T1 (**D**). Articular cartilage of HILT group shows at T0 an altered cell arrangement, a not well defined tidemark and low GAG amount; at T1, this group shows instead a regular surface, a high proteoglycan content, a good matrix organization. All the images are shown at the same magnification, as indicated by the scale bars. All the insets report some details of the images at high magnification.*

cartilage metabolism rendering it an ideal tool for the treatment of musculoskeletal diseases (17, 20).

In particular, the use of HILT provided good results by *in vitro* (21) and *in vivo* studies (17, 20, 24, 32). It is possible to hypothesize that HILT is able to provide clinical benefits, acting on deep body structures throughout a mechanical stimulus very close to the physiological loading. This effect would seem to be directly proportional to the laser emission intensity and inversely proportional to the pulse duration (data not shown). It is well known, that the applied energy acts with a photomechanical wave permitting to interact with the proteins of the extracellular matrix. These interactions provide a mechanical stimulus via the integrin network and the cytoskeleton, that in turn induce the activation of signalling pathways involved in the proliferation and differentiation processes of chondrocytes (33). This is in agreement with other studies, which emphasize the concept that the mechanical loading of cartilage tissue is an important requisite in various regenerative processes (34).

In general, the wide spectrum of parameters used by different Authors in the laser treatment such as wavelength, fluency, power density, pulse structure and treatment timing led to the publication of contrasting findings (35). The biochemical mechanisms underlying the effects exerted by laser treatment are for all these reasons incompletely understood.

In the current study, we investigated the clinical, histological and immunohistochemical effects of Nd:YAG HILT therapy on human cartilage lesions of the knee. The patients who received this therapy

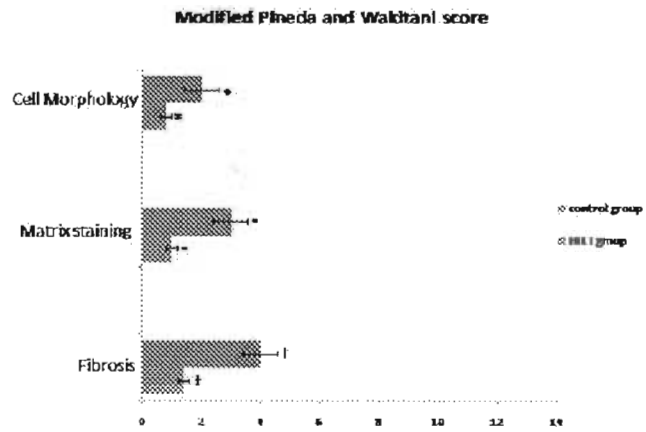

Fig. 4. *Modified Pineda and Wakitani score. Data are reported as Mean ± Standard Deviation (SD). Significant differences were observed for cell morphology, matrix staining and fibrosis for control versus HILT group (P= 0,031). The analyses were made with the Mann Whitney non parametric test; a t-test with the Welch correction was also performed.*

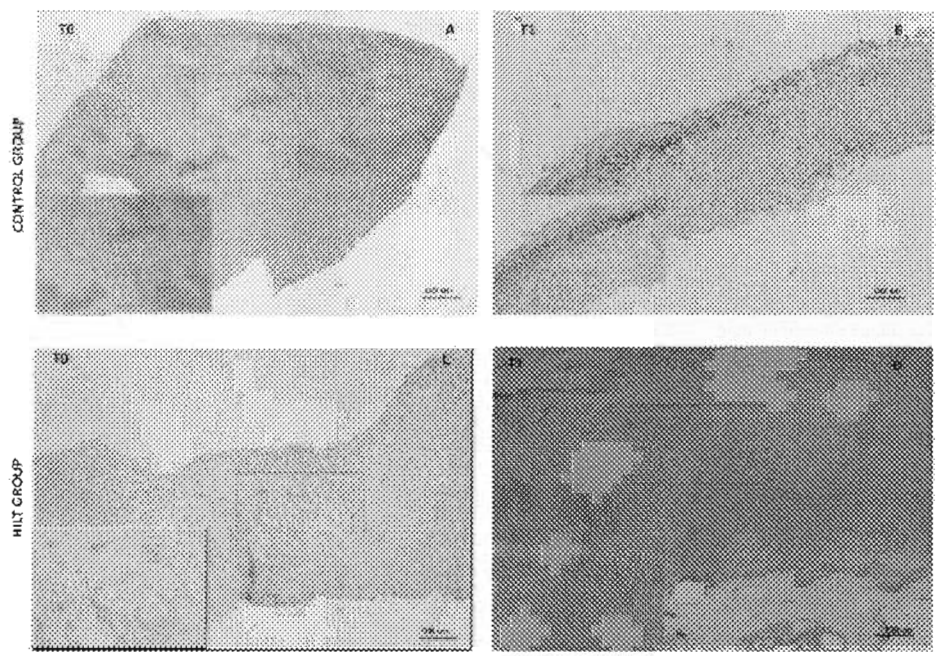

Fig. 5. *Immunostaining of type II collagen of articular cartilage sections in both control and HILT groups at T0 and T1. A slight positivity for type II collagen at extracellular level is evident in the control group at T0 (**A**) and the same expression is observed at T1 (**B**). A slight positivity is detected in HILT group at T0 (**C**), while an intense positivity is observed at T1 (**D**). All the images are shown at the same magnification, as indicated by the scale bars. All the insets report some details of the images at high magnification.*

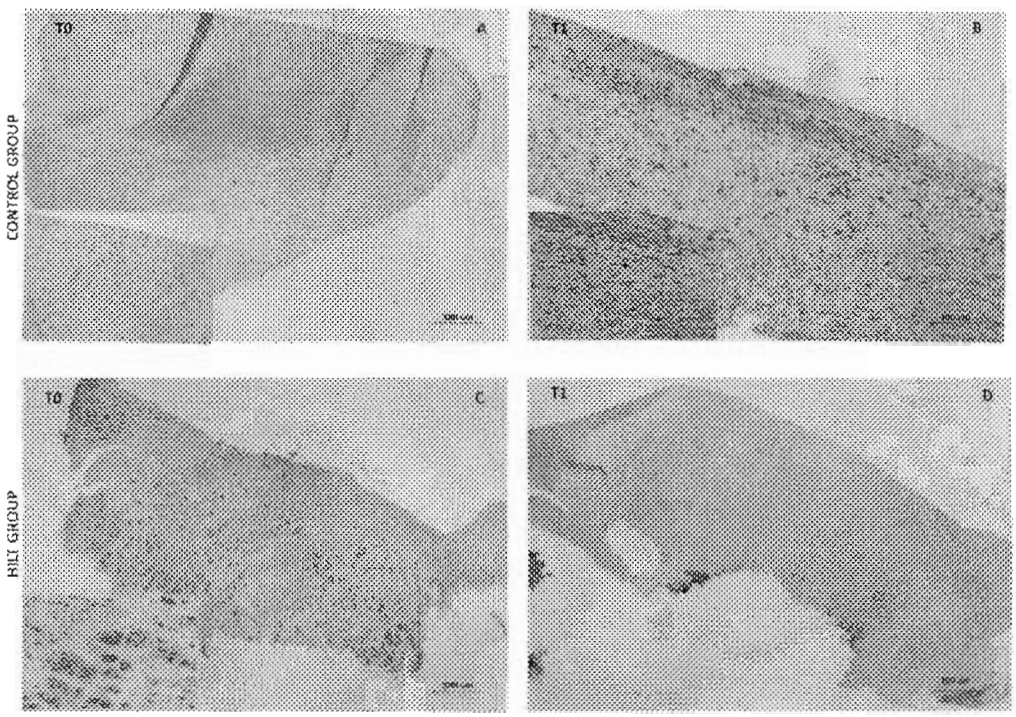

Fig. 6. *Immunostaining for type I collagen of articular cartilage sections in both control and HILT groups at T0 and T1. In control group, type I collagen is highly expressed at cellular level at T0 (**A**), a strong positivity is also evident at T1 (**B**). In HILT group, a positivity for type I collagen is observed at cellular level at T0 (**C**), while no positivity is evident at T1 (**D**). All the images are shown at the same magnification, as indicated by the scale bars. All the insets report some details of the images at high magnification.*

Table I. *Features of laser treatment.*

λ (nm)	PIF $[J/cm^3]^2$	Peak Intensity (kW/cm^2)	Total energy/day (kJ)
1.064	0.55	13±2	3±0.7

PIF: Pulse Intensity Fluence

showed in general a significant regeneration of articular cartilage in terms of a regular surface, a high proteoglycan content, a good integration with the adjacent articular cartilage. This positive contribution was particularly evident in the youngest subject who reported the lowest modified Pineda and Wakitani score (25, 26) and is correlated with the age of the patients. By contrast, a progressive filling with fibrous tissue of the cartilaginous lesions was detected in the control group at T1.

Table II. *Objectives and subjective scores based on cartilage lesions.*

Patients	Second look arthroscopy (days)	Age	Gender	ICRS T0	ICRS T1	Area lesion T0 (cm^2)	Area lesion T1 (cm^2)	CRA (0-12)	ORA (I-IV)
HILT 1	50	16	F	3	0	4.0	0	12	I
HILT 2	40	23	M	3	1	1.0	0.10	8	I
HILT 3	45	47	M	4	2	1.5	0.5	4	II
HILT 4	60	39	M	4	2	4.0	0.35	4	II
HILT 5	50	33	M	4	2	2.6	0.26	7	III
CNT 1	45	44	M	4	4	3.8	3.7	2	IV
CNT 2	50	38	M	4	4	1.5	1.5	0	IV
CNT 3	40	21	M	4	4	5.0	5.0	0	IV
CNT 4	60	18	M	3	4	2.3	2.2	0	IV
CNT 5	50	31	F	4	4	3.3	1.7	1	IV

*The data show the number of the specimens analyzed and are reported as Mean ± Standard Deviation (SD) of the following parameters: HILT: HILT patients; CNT: control patients; F: female; M: male; ICRS: International Cartilage Repair Society; CRA: Cartilage Repair Assessment; ORA: Overall Repair Assessment respectively at T0: time before laser treatment and T1: time after laser treatment. Significant differences are reported. * HILT versus control group ($P<0.01$) for ICRS at T1 and † HILT group at T0 versus T1 ($P<0.01$) for area lesion.*

Table III. *Histological scoring system.*

PATIENTS	AGE	GENDER	HISTOLOGICAL SCORE (0-14)
HILT 1	16	F	0
HILT 2	23	M	0
HILT 3	47	M	6
HILT 4	39	M	3
HILT 5	33	M	1
CNT 1	44	M	10
CNT 2	38	M	9
CNT 3	21	M	9
CNT 4	18	F	10
CNT 5	22	M	8

HILT: patients treated with laser therapy; CNT: patients who did not receive laser treatment, used as controls; F: female; M: male.

Immunohistochemical evaluations confirmed the good quality of the cartilaginous tissue regenerated in the HILT group, with a high pattern of expression for type II collagen a down-regulation of type I collagen.

The study shows some limitation due in particular to the low number of patients evaluated. However, it provides promising results on the use of HILT for the repair of chondral lesions, rendering it a possible non-invasive and therapeutic tool for clinical application.

ACKNOWLEDGEMENTS

Authors thank Prof. Julie Glowacki of Harvard School of Dental Medicine of Boston for her helpful advices in histological assessment and statistical evaluations. Thanks to Patrizia Rappini and Graziella Salmi for their assistance in the preparation of the manuscript and Luciano Pizzi for his technical assistance. This work was supported by grants from Progetto Regione Emilia-Romagna Università 2007-2009 "Regenerative medicine in osteo-articular diseases".

REFERENCES

1. Buckwalter JA, Rosenberg LC, Hunziker EB. Articular cartilage: composition, structure, response to injury and methods of repair. In Articular cartilage and knee joint function: basic science and arthroscopy. J.W. Ewing eds Raven Press New York 1990; p. 19.
2. Mankin HJ. Current concepts review: the response of articular cartilage to mechanical injury. J Bone Joint Surg (Am) 1982; 64:460-6.
3. Kuettner KE. Cartilage integrity and homeostasis. In Rheumatology. J. Klippel, P.A. Dieppe, eds Mosby International London 1998; p. 6.1.
4. O'Driscoll SW. The healing and regeneration of articular cartilage. J Bone Joint Surg 1998; 80:1795-1812.
5. Clarke HD, Scott WN. The role of debridement: through small portals. J Arthroplasty 2003; 18(1):10-13.
6. Takao M, Ochi M, Naito K, Uchio Y, Kono T, Oae K. Arthroscopic drilling for chondral, subchondral, and combined chondral-subchondral lesions of the talar dome. Arthroscopy 2003; 19:524-30.
7. Steadman JR, Rodkey WG, Rodrigo JJ. Microfracture: surgical technique and rehabilitation to treat chondral defects. Clin Orthop Relat Res 2001; 391:362-9.
8. Ritsilä VA, Santavirta S, Alhopuro S, et al. Periosteal and perichondral grafting in reconstructive surgery. Clin Orthop Relat Res 1994; 302:259-65.
9. Schindler OS. Cartilage repair using autologous chondrocyte implantation techniques. J Perioper Pract 2009; 19:60-4.
10. Kon E, Verdonk P, Condello V, Delcogliano M, Dhollander A, Filardo G, Pignotti E, Marcacci M. Matrix-assisted autologous chondrocyte transplantation for the repair of cartilage defects of the knee: systematic clinical data review and study quality analysis. Am J Sports Med 2009; 37:156-66.
11. Satija NK, Singh VK, Verma YK, et al. Mesenchymal stem cell-based therapy: a new paradigm in regenerative medicine. J Cell Mol Med 2009; 13:4385-402.
12. Giannini S, Buda R, Vannini F, Cavallo M,Grigolo B. One-step Bone Marrow-derived Cell Transplantation in Talar Osteochondral Lesions. Clin Orthop Relat Res 2009; 467:3307-20.
13. Beckerman H, De Brie RA, Bouter LM, Oostendorp RA. The efficacy of laser therapy for musculoskeletal and skin disorders: a criteria-based meta-analysis of randomized clinical trials. Phys Ther 1992; 72(7):483-91.
14. Basford JR: Laser therapy: scientific basis and clinical role. Orthopedics 1993; 16(5):541-7.
15. Hardie EM, Carlson CS, Richardson DC. Effect of Nd: YAG laser energy on articular cartilage healing in the dog. Lasers in Surgery and Medicine 1989; 9(6):595-1.
16. Spivak JM, Grande DA, Ben-Yishay A, Menche DS, Pitman MI. The effect of low-level Nd:YAG laser energy on adult articular cartilage *in vitro*. Arthroscopy 1992; 8:36–43.
17. Sobol EN, Baum OI, Shekhter AB, Guller A and Baskov AV. Laser-induced regeneration of cartilage. J Biomed Opt 2011; 16(08)80-2.
18. Zati A, Fortuna D, Valent A, Pulvirenti F, Bilotta TW. Treatment of low back pain caused by intervertebral disk displacement: comparison between high power laser, TENS and NSAIDs. Med Sport 2004; 57(1):77-82.
19. Verardi L, Mondardini P, Tanzi R, Briglia S, and Drago. Nd:YAG power laser in the therapy of the rectal adductor syndrome in athlete. Med Sport 2000; 53(4):343-50.
20. Lubich T, Mondardini P, Verardi L, Kanellopulu S, and Zoratti M. Power Laser in the early treatment and recovery of the injured athlete. Med Sport 1997; 50(1):71-83.
21. Monici M, Basile V, Cialdai F, Romano G, Fusi F, Conti A. Irradiation by pulsed Nd:YAG laser induces the production of extracellular matrix molecules

by cells of the connective tissues. A tool for tissue repair. In Biophotonics: Photonic solutions for Better Health Care. J. Popp, W. Drexler, V.V. Turchin, D.L. Matthews, eds Proc of SPI 2008; p.1.

22. Schultz RJ, Krishnamurthy S, Thelmo W, Rodriquez JE, Harvey G. Effects of varying intensities of laser energy on articular cartilage: a preliminary study. Laser Surg Med 1985; 5(6):577-88.
23. Fortuna D, Rossi G, Zati A, Cardillo I, Bilotta TW, Pinna S, Venturini A, Masotti L. Nd:YAG laser in experimentally induced chronic degenerative osteoarthritis in heavy line chicken broiler - pilot study. Proceedings of SPIE 2002; 4903:77-84.
24. Brittberg M, Winalski CS. Evaluation of cartilage injuries and repair. Bone Joint Surg Am 2003; 85(2):58-69.
25. Pineda S, Pollack A, Stevenson S, Goldberg V, Caplan A. A semiquantitative scale for histologic grading of articular cartilage repair. Acta Anat (Basel) 1992; 143:335-40.
26. Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, Goldberg VM. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg Am 1994; 76:579-92.
27. Bagnis A, Saccà SC, Iester M, Traverso CE. Cystoid macular edema after cataract surgery in a patient with previous severe iritis following argon laser peripheral iridoplasty. Clin Ophthalmol 2011; 5:473-76.
28. Lubart R, Friedmann H, Lavie R, Baruchin AM. A novel explanation for the healing effect of the Er:YAG laser during skin rejuvenation. J Cosmet Laser Ther 2010; 12(6):256-7.
29. Eltas A, Orbak R. Effect of 1,064-nm Nd:YAG laser therapy on GCF IL-1β and MMP-8 levels in patients with chronic periodontitis. Lasers Med Sci 2011; 26.
30. Bjordal JM, Couppe C, Chow RT, Tuner J, Ljunggren EA. A systematic review of low level laser therapy with location-specific doses for pain from chronic joint disorder. Aust J Physiother 2003; 49:107-15.
31. Brasseau L, Welch V, Wellis G, Tugwell P, Bie R, Gam A, Harman K, Shea B, Morin M. Low level laser therapy for OA and RA: meta analysis. J Rheumatol 2002; 27:1961-9.
32. Santamato A, Solfrizzi V, Panza F, Tondi G, Frisardi V, Leggin BG, Ranieri M, Fiore P. Short-term Effects of High-Intensity Laser Therapy Versus Ultrasound Therapy in the Treatment of People With Subacromial Impingement Syndrome: A Randomized Clinical Trial. Physical Therapy 2009; 89(7):643-52.
33. Monici M, Cialdai F, Fusi F, Romano G, Pratesi R. Effects of pulsed Nd: YAG laser at molecular and cellular level. A study on the basis of Hilterapia®. Energy for Health 2009; 03:26-33.
34. Patwari P, Lee RT. Mechanical control of tissue morphogenesis. Circ Res 2008; 1103(3):234-43.
35. Fortuna D, Paolini C, Ronconi L. Low Level Laser Therapy (LLLT): comparison between randomized double-blind clinical trials. Laser in Medical Science 2002; 17(4):A5.