

eBook

MLS® IN WOUND HEALING

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What is MLS®?

The MLS® Laser Therapy is born from ASA scientific research to overcome the limits of effectiveness of traditional laser therapy and merge the best of low and high power technology together, maintaining the safety.

MLS® (Multiwave Locked System) is a laser therapy that involves the use of two different emissions, combined and synchronised together: one with continuous/frequenced mode at 808 nm wavelength, the other with pulsed mode at 905 nm.

The mode of delivery and the proper balancing of the powers allow to exploit at the same time, and in complete safety, the positive effects of high power and low power laser, reducing treatment time and number of sessions.

MLS® pulse has been extensively characterized and its effects are well documented. MLS® has been clinically applied for the treatment of several pathologies, including shoulder pain, lumbago, carpal tunnel syndrome, etc.

Wounds and wound healing

Wound healing comprises the four phases of the repair process: haemostasis, inflammation, proliferation, and remodeling. In order for the tissue to heal successfully, all the four steps have to be completed properly, in regards of both qualitative and quantitative aspects, as well as the timing.

The different types of wounds can be classified in several ways, among which the main classification distinguish between acute and chronic wounds.

Acute wounds

Acute wounds are injuries that occur suddenly rather than over time. They can be divided into:

- Surgical wounds – they are incisions made purposefully by a healthcare professional and are cut precisely, creating clean edges around the wound. Surgical wounds may be closed (with stitches, staples or adhesive) or left open to heal. The healing process for surgical wounds is classified by their potential for infection.

- Traumatic wounds – they are injuries to the skin and underlying tissue caused by a force of some nature. They are typically defined as cuts, lacerations or puncture wounds. According to the severity and aetiology of traumatic wounds, treatment can either be simple or more extensive i.e. requiring surgical intervention to close the wound and stabilize the patient.

Chronic wounds

Chronic wounds are injuries that are not spontaneously evolving towards healing nor progressing through inflammation, proliferation, and remodelling phases. This condition represents a serious physical and psychological limit to the patient as well as being a burden for the health system. Many factors are contributing to grow the attention to chronic wounds, such as rising aging population, rising diabetic wounds due to changing lifestyle factors, and growing population with pressure ulcers. Ulcers are the most common types of chronic wounds. An ulcer is the breach of the continuity of skin, epithelium or mucous membrane caused by sloughing out of inflamed necrotic tissue. Despite treatment, many chronic ulcers fail to heal or persist for months/years and/or recur after healing, requiring additional advanced wound care therapies for adequate healing. Common forms of ulcers include:

- Decubitus ulcers, also known as bedsores. These ulcers are localized damage to the skin and/or underlying tissue that usually occur over a bony prominence because of pressure, or pressure in combination with shear and/or friction. The most common sites are the skin overlying the sacrum, coccyx, heels or the hips, but other sites such as the elbows, knees, ankles, back of shoulders, or the back of the cranium can be affected. Pressure ulcers most commonly develop in bedridden or confined to a wheelchair person. The healing of pressure ulcers may be slowed by factors such as: patient age, medical conditions (i.e. arteriosclerosis, diabetes or infection), smoking or medications such as anti-inflammatory drugs. Hospital-acquired pressure ulcers are concerning problems among healthcare professionals because they threaten the patients' quality of life and increase hospital costs. The rate of pressure ulcers in hospital settings is high: the prevalence in European hospitals ranges from 8.3% to 23% [1]. Pressure ulcers resulted in 29,000 deaths worldwide in 2013 up from 14,000 deaths in 1990 [2]. Option to treat pressure ulcers include pressure redistributing support surfaces, nutritional support, repositioning, wound care (e.g. debridement, wound dressings) and biophysical agents (e.g. electrical stimulation, electromagnetic fields). Nevertheless, reliable scientific evidence to support the use of these interventions is lacking.

- Diabetic foot ulcer, a major complication of the diabetic foot. Approximately 15–25% of individuals with diabetes develop a foot ulcer, of which an estimated 12% require lower extremity amputation [3].

Patients with diabetic foot ulcers are prone to infection and the healing process is complicated by diabetic neuropathy leading to chronic non-healing ulcers. These ulcers are a major health problem worldwide and have great impact at personal, professional and social levels, with high cost in terms of human and material resources.

Diabetic foot ulcers impose tremendous medical and financial burden on healthcare system with costs conservatively estimated as high as \$45,000 per patient [4]. Standard treatment includes wound cleansing, necrotic tissue debridement and, if necessary, treatment of infection, mechanical off-loading, management of blood glucose levels and wound dressing application.

A wide variety of advanced treatment for non-healing ulcer has been proposed, including hyperbaric oxygen therapy, skin grafting, VAC (vacuum assisted closure) and surgical management.

- Ulcers of the lower legs (VLU), a serious complication of chronic venous insufficiency. Ulcers of venous origin are a complex state depending on many pathological processes which lead to venous hypertension.

Venous ulcers occur in about 0.3% of the adult population in Western Europe and its incidence increase with age. [5-8] If reflux takes place in at least two venous systems, then ulcers will occur in 2/3 of patients [9-10].

Approximately 80% of VLU patients experience various types of severe pain including nociceptive and neuropathic pain [11]. Wound pain decreases the quality of life in patients. The ulcer treatment takes a long time and healed wounds often recur.

Only 50% of ulcers can be healed after 4 months [12], 20% remain open after 2 years, and 8% remain open after 5 years [13]. The recurrence of an ulcer, a year after total healing, occurs in about 6–15% of cases [14-16].

- Arterial ulcers – consequence of arterial insufficiency and less common than venous ulcers. They are caused usually by atherosclerosis, or, more rarely, thromboembolic or radiation damage. Arterial leg ulcers result from insufficient blood supply to the tissues. In patients with advanced stages of peripheral arterial disease (PAD), ulcers of the lower leg are common, due to reduced microcirculation in the extremities [17]. In 2010 about 202 million people had PAD worldwide. In the developed world, it affects about 5.3% of 45 to 50 years old and 18.6% of 85 to 90 years old. In the developing world, it affects 4.6% of people between the ages of 45 to 50 and 15% of people between the ages of 85 to 90 [18]. In the developed world, PAD is equally common among men and women while in the developing world women are more commonly affected.
- Traumatic ulcers originate after a trauma in a body area where skin was apparently healthy. Ulcer forms as the trauma provokes an disequilibrium in the vascular situation, unveiling a pre-existing deficiency. Commonly, the limb swells and this enlarges the wound and makes ischemia to progress. The treatment of these ulcers can be challenging, especially in elderly patients.

Current treatment for wound healing

Acute wound treatment

Treatment of acute wounds depends upon location and severity. General wound care may include the following:

- Controlling the bleeding – Identifying the source of bleeding and applying pressure (if applicable) or tools to make it stop.
- Cleansing the wound – General soap and water may be used on minor acute wounds. Saline solutions may be used on larger, deeper or more complex wounds.
- Debridement
- Dressing and/or closing the wound – Some wounds may require a health care professional to apply staples, skin adhesive, sterile strips or stitches to bring the wound edges together to close. Wounds may be left open to air or covered with dressings depending upon their location and severity.
- Antibiotics and other drugs – In some cases, antibiotics may be prescribed to thwart off infection. This is typical in wounds with a high risk of developing infection, as in those contaminated with debris. Medications for pain, swelling and other wound specific treatments (e.g. a tetanus shot) may also be recommended.

Chronic wound treatment

Chronic wounds, which typically result from medical conditions, nutritional deficiencies, infections, and metabolic disorders, involve intensive and time-consuming treatments. Treatments in patients with chronic wounds usually consist in debridement and skin grafts. Other therapeutic approaches may include:

- **Advanced wound dressing** – Alginate, film, foam, hydrocolloid and hydrogel dressings can regulate the wound surface by retaining moisture or absorbing exudate, so protecting the wound base and tissue surrounding the wound.
- **Negative pressure wound therapy** – this therapeutic technique uses a vacuum dressing to promote wound healing. The therapy involves the controlled application of sub-atmospheric pressure to the local wound environment, using a sealed wound dressing connected to a vacuum pump.
- **Hyperbaric oxygen therapy (HBOT)** – HBOT is a treatment designed to increase the supply of oxygen to wounds that are not responding to other treatments. HBOT involves people breathing pure oxygen in a specially designed compression chamber (e.g. those used for deep-sea divers suffering pressure problems after resurfacing).
- **Physical therapies** – extracorporeal shock wave therapy, whirlpool, electrical stimulation, ultrasound, magnetotherapy, low-power laser and compression therapy are physical therapy modalities that have been used to enhance wound healing. These modalities are used as adjunct treatments that, when appropriate, may help shorten the length of treatment and reduce patient suffering.
- **Biologics** – The use of platelet-rich-plasma (PRP), different types of growth factors and bioengineered cellular approaches are applied to augment or modulate the wound healing process itself.

Why MLS® in wound healing

Basic research: *in vitro* studies

Wound healing is a complex and multistep process, crucial for the survival of the organism. It consists of four partially overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling. A proper sequence and timing of events is essential for a successful repair. In order to evaluate and understand the effects of MLS® emission on the evolution of the healing process, *in vitro* and *in vivo* studies have been performed and are still ongoing in our laboratories.

Proteomic studies were carried out on myoblasts: C2C12 cell line, a model of muscle satellite cells. These cells can repopulate hypotrophic and damaged muscles, thus playing an important role in muscle repair.

The results of these studies show that MLS® treatment induces an increase of NLRP 10, a protein with anti-inflammatory action. NLRP 10 inhibits the activity of caspase 1 and PYCARD protein complex, which promote the synthesis and release of the inflammatory cytokines interleukin-1 β (IL-1 β) and interleukin 18 (IL-18). Therefore, NLRP 10 inhibits the production of pro-inflammatory interleukins IL-1 β and IL-18, reducing inflammation [19]. The anti-inflammatory activity of MLS®, mediated by NLRP 10 production, can be usefully exploited in the treatment of wounds and ulcers to control inflammation. The decrease in inflammation leads to a normalization of vascular function and thus to a decrease of edema. Obviously, the decrease in inflammation and edema results in a decrease of pain symptoms that are frequently present in patients with wounds or ulcers.

Further results of the above studies revealed increased expression and/or modulation of several proteins involved in cell cycle regulation, cytoskeleton organization and differentiation, clearly indicating a cellular response of anabolic type. It was also observed a significant increase in proteins able to bind ATP, which could be related to an increased ATP availability. In other words, the increase in proteins that bind ATP allows to better exploit the amount of ATP available to carry out biological processes. Enhanced anabolism and energy metabolism can better sustain the repair processes [19].

A highly significant result is the increase of protein phosphatase-1 (PP1). This protein is involved in many important processes, including the regulation of glycogen metabolism and the process of relaxation/contraction in smooth muscles. Moreover, following the MLS® treatment, a significant increase (approximately 80%) of the total phosphatase activity was observed [19, 20]. Phosphatases are enzymes which play a crucial role in biological functions and virtually control basic processes at cellular level, including metabolism, gene transcription, cell cycle, cytoskeletal organization, stability and interaction of proteins, adhesion, migration and apoptosis. The last two (migration and apoptosis) are strongly involved in the healing process and regulate the succession of different cells populations (immune cells, endothelial cells, fibroblasts) at the wound site.

Other studies carried out on myoblast cultures showed that the MLS® treatment can affect the production of molecules (collagen, metalloproteases) involved in extracellular matrix turnover [21]. The production of new extracellular matrix and its assembling is a fundamental step in the repair process, in particular during the remodelling phase.

MLS® treatment can improve the healing of wounds and ulcers not only acting on repair mechanisms but also decreasing the survival of contaminating microorganisms, which frequently colonize injured tissues. Moreover, it alters their inflammatory potential as demonstrated by our studies on *Candida* spp. (*C. albicans*, *C. glabrata*, *C. parapsilosis*), [22].

More recent studies on an in vitro model of wound healing (scratch assay on fibroblast monolayers) exposed to MLS® emission, showed that the treatment increases fibroblast migration and fasten the healing of the scratch [23].

For future studies on tissue repair, new in vitro models of sutured wounds, based on tissue cultures, have been developed in our laboratories.

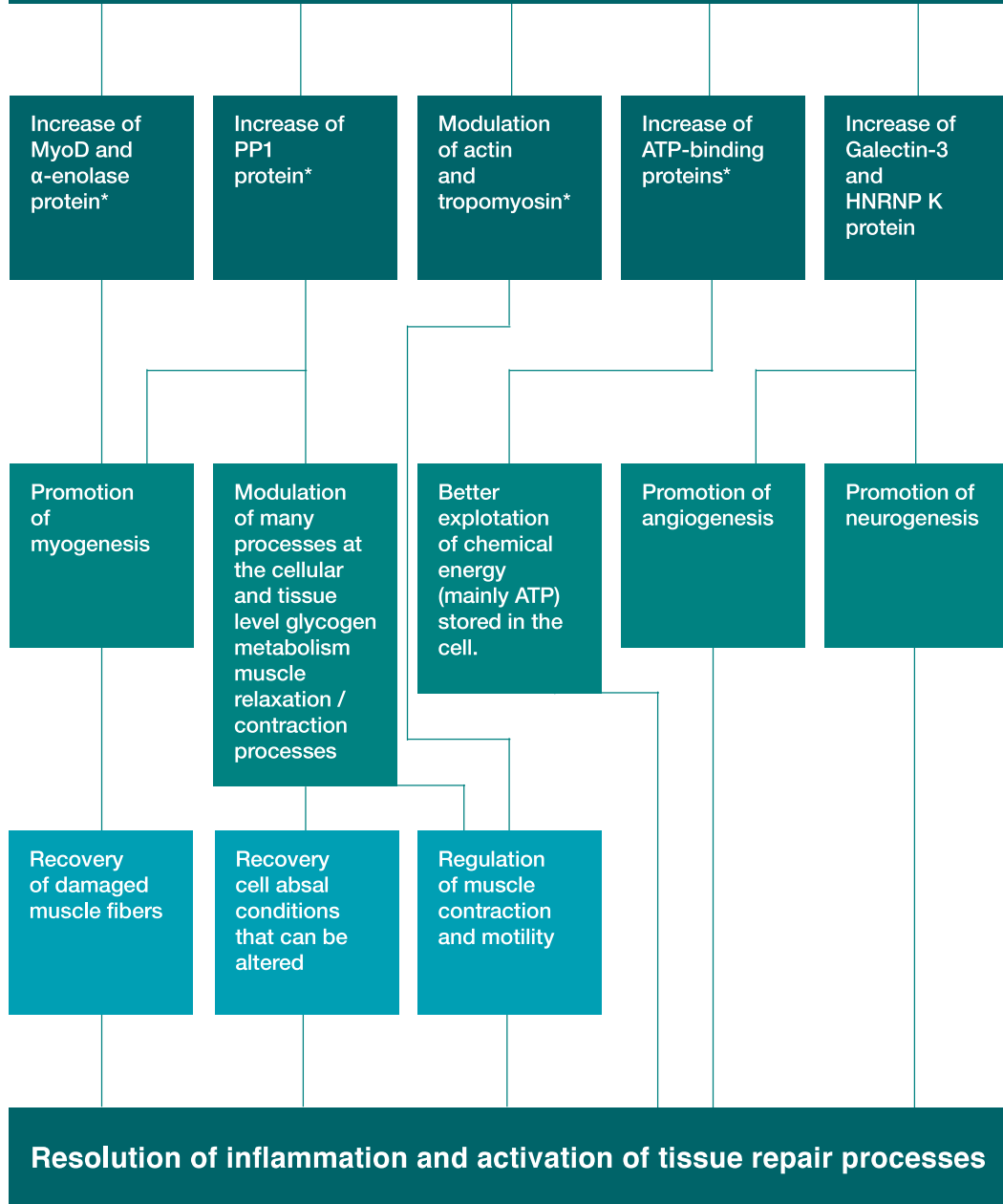
Basic research: *in vivo* studies

In order to study the repair processes, we developed also a new in vivo model of sutured/unsutured wound based on the leech. Preliminary studies, recently carried out using this model, showed that the wounds treated with MLS® healed more quickly than the untreated control and the quality of the scar tissue was improved by the treatment (results not yet published).

MLS® features make it a valid tool for promoting wound healing. Specifically:

- MLS® acts on inflammation thanks to modulation of NLRP10 that inhibits the production of pro-inflammatory interleukins IL-1 β and IL-18;
- MLS® can enhance cell energy metabolism by increasing the production of ATP-binding proteins and ATP availment;
- MLS® can favour anabolic processes;
- MLS® can favour fibroblast migration;
- MLS® can affect the ECM turnover;
- MLS® can affect survival and inflammatory potential of contaminant microorganisms.

MLS® Laser Therapy



* MONICI M, CIALDAI F, ROMANO G, CORSETTO PA, RIZZO AM, RANALDI F. (2012) Effect of IR Laser on Myoblasts: Prospects of Application for Counteracting Microgravity-Induces Muscle Atrophy. Microgravity Science and technology; 25(1):35-42; *MONICI M, CIALDAI F, RANALDI F, PAOLI P, BOSCARO F, MONETI G. CASELLI A. (2013) Effect of IR Laser myoblasts: a protemic study, Molecular Biosystems. DOI: 10.1039/c2mb25398d

Clinical evidences of MLS® in wound healing

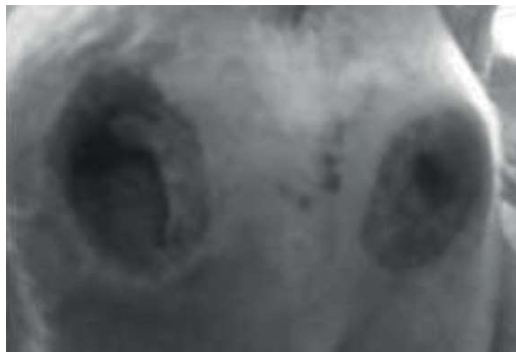
Animal data

Dog pressure Ulcers

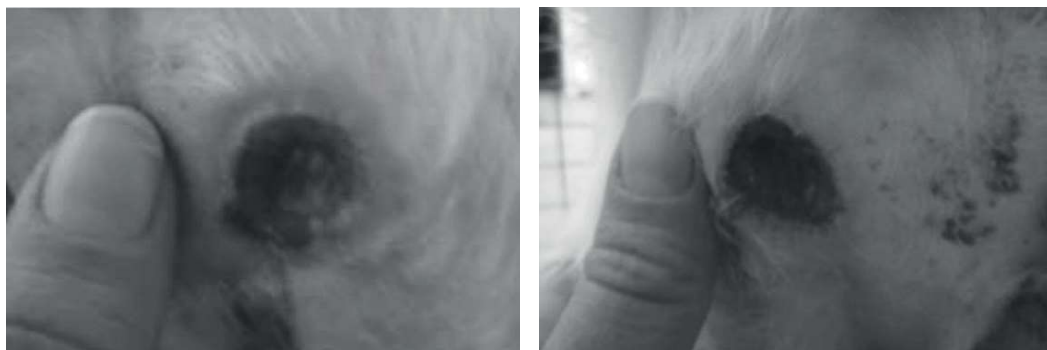
Patient: 8 yo male mixed breed dog, weighing 13 kg, was presented in the clinic after the right hemilaminectomy at T12 to T13 performed for intervertebral disk disease. The examination revealed a non-ambulatory paraparesis and bilateral tuber ischium stage III pressure ulcers developed because the dog was allowed to drag itself only on his front paws. The wounds were full thickness, 2,1 x 2,1 cm on the left tuber ischium, 1,9 x1,9 cm on the right tuber ischium, but bone was not exposed [24].

MLS® Laser Treatment: MLS® and topical wound medications two times a day. Administered dose was = 1,76 J/cm².

Treatments were given for 12 consecutive days and pressure sores were measured twice, once at the beginning of the treatment and at the 12th day of the treatment.



Decubitus ulcers before MLS® treatment.



Ulcers after MLS® treatment.

At the second measurement, the dimensions of the ulcers were 1,2 x 1,2 cm for the left ulcer and 1,3 x 1,3 cm for the right ulcer. The depth of the wounds was diminished. The dimensions of the wounds were reduced by 42,8% and 31,6% respectively in 12 days.

Dog with bedsores

Patient: 4 yo dog. The dog had a disk herniation in the lumbar tract, between L1 and L2. This situation caused paresis, that led to bedsores.

Treatment: the dog had daily antibiotics and disinfection of the injuries. MLS® Laser Therapy was applied on alternate days for five times.



Before MLS® laser treatment



After second MLS® laser treatment



After fifth MLS® laser treatment

Dog traumatic wound

Patient: Female 9 yo dog presenting a contused and lacerated wound originated by the impact with a metal object while running. Curettage was performed as, after primary suture, the skin was necrotic.



Before MLS® laser treatment



After 15 days from the end of the MLS® laser treatment

Dog traumatic injury

Patient: male 6 yo dog. His left foreleg was severely injured, probably because of a trap. The injury was lacerated and contused. The edges were edematous and cold.

Treatment: after surgical debridement, antibiotics and anti-edema treatment, the patient started the MLS® therapy sessions.



Before MLS® laser treatment



After MLS® laser treatment

Dog traumatic wound

Patient: male 4 yo dog, injury during hunting, probably due to hitting a stone or a branch. After surgery, the wound reopened.

Treatment: the dog received 15 MLS® laser treatment sessions.



Before MLS® laser treatment



After 15 MLS® laser treatment sessions.

Horse traumatic wound

Patient: female horse found with a wound in the sizing zone, on both sides, probably as a result of biting. Antibiotics, serum tetanus and anti-inflammatory drugs were administered.

At the time of the visit to the clinic, the horse had a subcutaneous sizing emphysema and swelling of the chest, neck and face. The wound was exudating and necrotic tissue was present. Trachea and jugular vein were exposed.

The wound was protected by a dressing that was changed daily. The prescribed treatment was antibiotic, anti-inflammatory and anti-edema medications.

MLS® laser treatment: From day 5, daily MLS® sessions up to day 14, then every other day until day 24. After 89 days, the wound was almost completely closed. Thanks to MLS® treatment, no keloid formation happened and an optimal repair was observed.



Before MLS® laser treatment



During MLS® treatment applications

After 89 days from injury

Parrot with self-inflicted wounds

Patient: rose-breasted Cockatoo with self-inflicted wounds. There was no significant improvement after a month of recovery and traditional therapies.

MLS® laser treatment: three times a week. The applications were carried out with the point-by-point technique along the edges of the wound and in concentric scanning on the wound itself. For the first two sessions, the applied dose was= 2,05 J/cm²



Before the first MLS® treatment

For the following sessions, the applied dose was=2,09 J/cm²



After 3 weeks of treatment



After 25 days from injury



Complete healing at the end of
MLS® therapy

Dog with bite wounds

Patient: the dog was attacked by a big size dog and was bitten several times in the lower back, hips and legs. He had deep lacerations with plenty of tissue loss and surgery was performed to try to close the wounds to the sides and to apply a drainage. The massive tissue loss due to the trauma and to the subsequent surgical curettage, made the complete closure of the lesion impossible. The wounds had post-surgery dehiscence.

MLS® laser treatment: 3 times a week up to 11 sessions

When not submitted to therapy, the dog had topical treatment and was bandaged to prevent contamination of the wound.

Healing of the left side wound almost complete and right side wound complete after the last session of MLS® Laser Therapy.



After surgery and drainage application



After first MLS® treatment



After second MLS® treatment



After eight MLS® treatment



At the end of the treatment

Canary with feather loss

Patient: 5 yo canary. The bird was brought in for an examination following the loss of feathers on the wings that had started approximately 4 months prior.

At the examination, the general conditions were considered good except for a serious case of feather loss on the wings, both on the upper and lower surface.

MLS® laser treatment: MLS® Laser Therapy once a day for 15 days. After three days, there is already a growth of the first feathers with further growth until the fifteenth day.



Before MLS® laser treatment



Growth of feathers after the first MLS® laser treatment

Fancy rat with surgical wound

Patient: 2 yo fancy rat: A benign subcutaneous 5 cm diameter lipoma has been removed but on the 3rd day after surgery he opened the surgical wound with his mouth.

MLS® laser treatment: Beside post-op pharmacological therapy, MLS® applications have been performed on the wound twice a day. After 3 days, the animal could be discharged from vet hospital, and continued therapy sessions every other day. The wound quickly improved and treatments ended 9 days after dehiscence, when the lesion had been completely closed by scar tissue.



Before MLS® treatment



At day 9 - end of treatment

Cat with traumatic wound

Patient: 3 yo cat with traumatic wound, hit by car.

MLS® laser treatment: every 3 days



Before MLS® treatment



After 6 treatments



After 10 treatments

MLS® indications in wound healing

MLS® Laser Therapy can be applied for the treatment of:

- ▶ Vein ulcers
- ▶ Artery ulcers
- ▶ Diabetic ulcers and due to vasculitis
- ▶ Wounds
- ▶ Surgical wounds
- ▶ Actinic ulcers
- ▶ Decubitus ulcers
- ▶ Traumatic ulcers

Table 1 – Examples of ulcer type and the correspondent MLS® treatment expected results.

ULCER TYPE	EXPECTED RESULT
Vein ulcer	Excellent possibilities of success
Diabetic ulcer and due to vasculitis	Lower possibilities of success. Variable case by case.
Artery ulcer	Few possibilities of success. Frequent relapses.
Acnitic ulcer	Excellent possibilities of success
Decubitus ulcer	Excellent possibilities of success, if properly cleaned
Traumatic ulcer	Excellent possibilities of success

How to use MLS® in wound healing

Before starting the treatment, the wound must be debrided and cleaned by any trace of necrotic tissue, pus or cream by means of surgical curettage or proper gel. In fact, surface scab obstructs local laser treatment, while necrotic tissues make infections easier and obstruct regenerative and healing processes. Curettage has also the aim to remove blood clots that could obstruct ulcer re-epithelialization. Bleeding should be stopped before starting with the laser treatment. After curettage, if the wound is infected, it must be cleaned with saline solution or hydrogen peroxide.

MLS® treatment should be applied from the bottom to the top of the lesion, covering the whole area, starting from the wound border and treating 2-3 cm of healthy tissue.

Recommended dose: 1-4 J/cm².

Doses below 1 J/cm² could be insufficient to promote the biostimulation, whereas doses above 4 J/cm² could have a negative effect (e.g. inhibition instead of stimulation of cell growth). The device parameters must be tuned considering the extension of lesion surface.

Treatment frequency: 3 times a week, or according to wound medication, until complete healing. If after the first four treatment sessions, there are no significant results, it is necessary to reconsider the diagnosis and the treatment protocol.

Application modality

- **Standard lens - Ø 2 cm - 3 cm²:** manual scan over the damaged area (+ 2-3 cm of healthy tissue). If using the handpiece, this should not be put in contact with the wound tissue, but rather kept at 0,5-1 cm.
- **Charlie - Ø 5 cm - 20 cm²:** fixed points to cover the whole lesioned area (+ 2-3 cm of healthy tissue).
- **Robotized head:** automatic scan to cover the whole lesion area. For dosing purposes, it must be considered that energy dispersion due to the distance between the probe and the skin is about 20%.

Parameters setting

- Select ulcer/wound dimensions in cm²
- Intensity =100%
- Frequency =1500 Hz
- Set the parameter "Time" to deliver an energy dose between 1 and 4 J/cm²

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