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Impact of laser therapy on the healing processes

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1. Abbreviations

ATP	Adenosine triphosphate
ALP	Alkaline phosphatase
CFU	Colony forming units
Ga-Al-As laser	Gallium- aluminium-arsenide laser
HeNe laser	Helium Neon laser
JAK/ STAT	Janos kinase signal transducer and activator of transcription
MRSA	Methicillin Resistant *Staphylococcus aureus*
NO	Nitric oxide
PROM	Passive Range of Movement (of a joint)
ROS	Reactive Oxygen Species
VEGF	Vascular Endothelial growth factor

2. Abstract

The use of treatment with laser therapy in the veterinary field has grown tremendously during the last few years. Laser therapy is used to treat a wide range of conditions both in humans and animals including the use on wound and bone healing, the treatment of bacterial infections in post-surgery wounds and to impact the processes during inflammation and pain.

In the literature it has been shown that laser therapy can accelerate the healing of wounds and positively impact the organization of cells and their products. Different doses of laser were investigated and in most cases a lower dose gave better results, if it was high enough to stimulate the cells. This was true both for cutaneous and oral wounds and in both healthy animals and rats with diabetes mellitus.

Regarding the bacterial studies the results differed a lot if they were carried out *in vitro* or *in vivo.* Different kinds of bacteria reacted differently on *in vitro* application of laser but all the cases where laser was applied *in vivo* showed decreased growth of the bacteria and improved healing of the animals used in the study.

When applying laser therapy on broken bones in living animals or osteoblasts *in vitro*, the literature review showed that the proliferation is stimulated and that the mechanical properties are overall better than if no laser treatment was carried out.

Laser therapy has been shown to reduce inflammation and pain in many different ways. The laser impacts several inflammatory mediators and gene expressions and thus cause decreased inflammatory responses in the body. This is closely linked with the sensation of pain and the studies carried out on laser treatment on it show promising results.

Scientific studies are being carried out at the moment for the use of laser therapy both in companion animals and humans. More research is needed to fully understand all the functions and application areas in the developing field of laser therapy.

3. Introduction

The aim of this literature review is to understand the function of laser therapy both on cellular level and on tissue level. The focus is on the particular effects of laser treatment on wound healing, microbial growth in wounds, bone repair processes and the inflammation cascade. On the cellular level the focus is on signaling pathways and the inflammatory mediators and their effects in the body.

Most of the earlier academic studies regarding laser therapy concern either the use of laser on experimental level (on rats or mice) or case studies and trials with human patients. With time and as the technology becomes cheaper and easier to use, laser therapy gets more and more common in everyday veterinary medicine and studies are now being carried out for companion animals as well.

Light as therapy method have been used for hundreds if not thousands of years. From the use of sunlight in medicine to different kinds of visible light with light bulbs and, as technology developed, with the help of modern technique as for example laser therapy.

Many times laser therapy is used together with other treatment such as pharmacological, surgical or physiotherapeutic methods. Studies in several different species have shown that laser treatment can accelerate wound-healing, impact the growth of bacteria both *in vitro* and *in vivo* and affect inflammation and pain condition. Other terms of use are after burns, snakebites and for a wide range of musculoskeletal conditions (Riegel & Godbold, 2017).

The name laser is a short, common name for phototherapy, the use of light in rehabilitation and therapy. Many different types of laser are in use today; the most common one for therapeutic purposes is the low-level laser therapy. Another name for it is cold laser, to differentiate them from the high-level laser lights used for surgery that will destroy the cells (Millis & Levine, 2013).
Other names of laser therapy include photobiomodulation, low power laser and laser irradiation.
Depending on the location, dose (in joules), power density (mW/cm^2) and wavelength (nm), the laser therapy can be given in either continuous or pulsatile duration. (Bjordal et al, 2003)
In this report, the name laser or laser therapy will mainly be used.

4. Laser technology

Laser therapy does not work by thermal effect and the laser wavelength used for therapeutic treatments barely changes the temperature of the site at all. The action of laser in the cells is a photochemical reaction. Light makes a shift in the redox potential in the membrane and increases the oxidation value. Depending of the growth stage of the cell, it will react different on the laser light. Cells with a low intracellular pH show a greater response to laser light than cells with a stable and normal pH that react less or not at all. Laser light lead to the increase of ATP in the cells and this provides the energy needed for the cells to activate the normal metabolic function (Jare et al, 2017).

One of the most important parameters in clinical practice is the treatment dosage (Rezende et al, 2007). It will be evaluated under each relevant chapter.

4.1 Types of lasers

Laser machines emit a flow of photons, which starts in the laser unit with the activation of electrons (Millis & Levine, 2014). The types of laser diodes used for therapeutic methods are HeNe, GaAs and Ga-Al-As (Jang & Lee, 2012).

The two most common laser devices in veterinary medicine are the HeNe and Ga-Al-As lasers. A HeNe laser is a diode with the medium Helium and Neon. Gallium- aluminum arsenide lasers use the medium gallium, aluminum and arsenide.

The difference between the two lasers is that Ga-Al-As laser works on the photochemical response in the cell membrane while the HeNe laser impacts the respiratory chain in the cell (Fahimipour et al, 2016).

4.2 Wavelength used

Light is emitted in waves, where the distance between the same points in two of each other following waves are the wavelength. Wavelength is used to differentiate different types of light from each other. Different colors of light have different wavelengths.

During research a wide span of wavelengths have been used to find the optimal wavelength for different conditions. Different wavelengths have different absorption and scattering in different tissues and there can be several wavelengths used depending on where and why the laser therapy is applied (Riegel & Godbold, 2017).

Wavelength spectrum of visible light goes from around 380 to 780 nm. The wavelength used for laser therapeutic purposes is in the span around 405 to 980 nm (Millis & Levine, 2013).

4.3 Techniques

Depending on where the problem is located, laser therapy can be applied with contact to the skin or in a non-contact way. Depending on the type of laser, the penetration will be deeper or more superficial. Contact application is suggested for musculoskeletal problems and other conditions deeper in the thoracic or abdominal cavity as well as inner and middle ear problems. For wound healing, infections in the treated area and intraoperative treatment a non-contact application is used (Riegel & Godbold, 2017).

5. Wound healing and tissue repair

The healing process starts right after an injury and consist of several steps and many different kinds of cells are involved (Rezende et al, 2007). The different stages of the healing process are started by a release of several compounds such as cytokines, growth factors and also by low weight serum proteins. These are released from the blood vessels and the platelets that are injured (Jere et al, 2017). The steps of healing include blood clotting, inflammation, formation of granulation tissue, collagen synthesis, epithelization and finally the tissue remodeling. If the area is not in ideal conditions regarding the inflammatory responses, angiogenesis or in the differentiation of the cells it can cause delayed or even defect healing (Riegel & Godbold, 2017). Many of these steps can be influenced by laser therapy (Rezende et al, 2007).

5.1 Laser therapy on wounds

Laser therapy has been proven to induce cell proliferation, enhance cell division, increase ATP synthesis in the cells and promote nucleic acid production (Reddy et al, 2001).

The purpose of laser therapy when it comes to tissue repair is to optimize the healing conditions to be able to achieve as good results as possible. It can enhance the cellular processes they are programmed for, but it can never come up with or change the programs of the cells. This can mean to stimulate the cells, but sometimes the action may be by not interfering with the ongoing processes (Riegel & Godbold, 2017). Low doses of laser light can promote changes in the cellular processes which may lead to increased proliferation of fibroblasts, the granulation tissue may form faster, promotion of collagen synthesis and even the epithelization can start earlier (Rezende et al, 2007).

5.1.1 Wound in rats

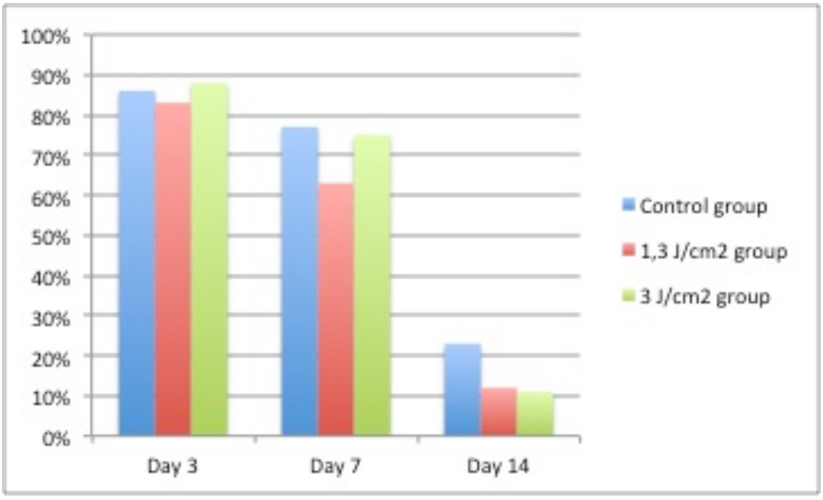
In a study made by the above-mentioned Rezende et al, 2007, rats receive a wound on their back. They were grouped according to the table 1.

Table 1. Grouping and treatments of rats in an experiment by Rezende et al, 2007 where the rats recived wounds on their backs which were treated with laser according to the table.

	Dose	Output	Time
Control, no treatment	0	0	0
Laser, low dose	1,3 J	60 mW	25 sec
Laser, higher dose	3 J	60 mW	56 sec

The groups receiving laser treatment were treated directly after the wound intervention. There were no antibiotics given and the wounds were left uncovered to heal by secondary intention.

Figure 1. Diameter of the wounds on the back of the rats compared to the original size*, in the experiment by Rezende et al, 2007.*



The figure shows the diameter of the wounds, when compared with the original size being 100%. Both laser treated groups showed similar repair processes. The largest difference was between the 1,3 J/cm^2 group and the control group after 7 days, which can be seen in the above figure 1.

At day 3 the histological results in all groups were similar with the exception of the characteristics of the deep dermis layer of the wound. In both laser treated groups the observation showed re-formed capillaries, fibroblasts and small collagen fibers.

After 7 days the control group still had parts where the epidermis was not recovered, compared to the laser groups that were both recovered by this time. Fourteen days after the intervention the epidermis was totally healed in all groups (Rezende et al, 2007).

5.1.2 Wound healing at different wavelengths

Different wavelengths can influence the tissues differently and Al-Watban et al, 2007, examined this phenomenon in a study. They examined the closing percentage of a surgically made wound on the back of rats with induced diabetes mellitus. In the study there were two control groups, one with non manipulated rats and one with induced diabetes mellitus, both of which received the same kind of wounds as the rats treated with laser. The different laser wavelengths examined was 532 nm, 633 nm, 810 nm and 980 nm. Power output were 143, 140, 200 and 200 mW respectively. Spot size was 7 cm^2 for the smallest (532 nm) wavelength and 9 cm^2 for the other three. The power densities were 20.4, 15.56, 22.22 and 22.22 mW/cm^2 for the same order of lasers as described before. Time used for the treatment varied between the laser wavelengths but the dosages were 5, 10, 20 and 30 J/cm^2. Each wavelength group had subgroups with these different dosages. All wounds were treated 3 times a week until total closure of the wound and the time until this point was measured.

The results of the study were presented in curves depending on the size of the wound and the mean slope value in mm^2/day was used for evaluation. The wounds on the non-diabetic rats healed faster than the diabetic control, as the authors expected. Their results are not evaluated here.

These are shown in the table 2 below.

Table 2. Results (mean slope value in mm^2) of the wound healing processes in diabetic rats at different laser wavelengths from a study by Al-Watban et al, 2007.

	532 nm	633 nm	810 nm	980 nm
D-Control	3.636	3.636	3.636	3.636
D- 5 J	4.563	5.6767	4.5926	4.415
D- 10 J	4.735	5.9756	4.5449	4.629
D- 20 J	4.825	5.5866	4.2212	4.475
D- 30 J	4.877	4.637	4.1081	4.107

When the % of wound healing acceleration was calculated the most effective laser treatment had a healing acceleration of 38.44%. It was the 633 nm wavelength and with the dose of 10J. Therefore, the authors concluded that this was the optimal dose in their study (Al-Watban et al, 2007).

5.2 Vascularization

For a wound or injury to heal the neovascularization or revascularization is of large importance. It will transport the materials needed for the rebuilding of the structure and the removal of the rest products. There are two different kinds of mechanisms that lead to neovascularization, these are angiogenesis and vasculogenesis. Angiogenesis means a new vessel is formed from an already existing network of capillaries and vasculogenesis is when the blood vessels are formed *de novo* from endothelial progenitor cells.

Laser therapy has been shown to have an important beneficial effect on both angiogenesis and vasculogenesis. The laser treatment is able to increase specific growth factors, for example the vascular endothelial growth factor (VEGF) that is controlling the neovascularization. Without this, the wound will not heal (Moon et al, 2018). Laser therapy has been used to promote the cells normal function and may be used on non-healing skin ulcers (Lau et al, 2015).

When comparing the blood circulation in a skin flap treated with either laser therapy, bone marrow derived stem cell or a combination of both the combined group showed significant higher blood flow than all the others. This indicates that laser therapy might be a well worth complement after injuries or trauma involving loose skin flaps (Moon et al, 2018).

5.2.1 Impact of laser treatment on vascularization

In the earlier mentioned study by Moon et al, 2018 they investigated the survival of the skin flaps on mice with different medical techniques.

The mice were divided into different groups; control, laser treated group, bone marrow derived stem cells treated group and a combination group.

Under anesthesia a skin flap was made on the back of the animals. A silicone sheet was put under the flap to avoid contact with the tissue below.

The light used gave wavelength with 850 nm and the energy dose given was 3,84 J/cm^2. Laser treatment was given immediately after surgery and thereafter on day 1, 2, 3 and 4. The final result was evaluated at day 7 after surgery by measuring both the ischemic or necrotic area and the viable areas of the skin flap. The control group had 43 ± 18% survival rates of the skin flap area, bone marrow derived stem cells group had 59± 23%, the laser group by itself had 54± 19% and the combined group with both laser and bone marrow derived stem cells injected into it had a survival of 71± 23%.

The histological picture in the control group revealed a large number infiltrated macrophages and lower collagenesis compared to the combined group and therewith less inflammation. Healing at day 7 had progressed longer in the combined group than the others histological speaking, with a close to normal structure (Moon et al, 2018).

5.3 Wound healing at cellular level

There are many factors that are thought to influence the total amount of collagen deposited in an area during wound healing. The factors can be both internal such as the activity of the cells and from external sources such as drugs or exposure to laser light (Medrado et al, 2008).

A study with laser therapy treatment on rats reveled that the stimulation of skin fibroblasts made them produce more matrix collagen than normal. Laser treated wounds showed better tensile strength compared to non-laser treated wounds. Also the procollagen mRNA levels were elevated in the cutaneous wounds treated with laser (Reddy et al, 2001).
It has been proved that wounds in rats made during experiments had more collagen deposited in them if they were treated with laser light, compared to the control group which did not receive any laser treatment (Medrado et al, 2008).

5.3.1 Organization of fibers

The study by Medrado et al, 2008 made a comparison of the amount of collagen present in the wound tissue of rats. Laser treatments were with 670 nm, 9 mW, 1J/cm^2 and given every other day until the total dosage reached 4 J/cm^2. Silica was injected into two groups to promote fibrosis. The evaluation was semi qualitative and graded 0=absent, 1= mild presence, 2= moderate presence and 3= marked presence of collagen. The results are shown in table 3 below (Medrado et al, 2008).

Table 3. Evaluation of collagen amount, graded 0-3, in a wound 10 days after the creation. The comparison is between different treatment groups in a study by Medrado et al, 2008.

Amount of collagen 10 days after wound intervention

	Median	**Lower value**	**Upper value**
Control	1	1	1.75
Laser	2	2	2
Silica	1	1	1.75
Laser + silica	2	2	2.75

Rezende et al, 2007 has shown that when it comes to the organization of the healing tissue and the fiber patterns it varies between the doses of laser. They used rats as models and created a wound on their backs. After treating the wound with laser therapy with the doses of 1.3 and 3 J/cm^2 there was a clear differences between the groups at day 7. The group with 3 J/cm^2 showed less organized connective tissue layer than the lower dose group. The 1.3

J/cm^2 presented a higher number of fibroblast cells and more newly formed capillaries than the 3 J/cm^2 group.

A control group, which did not receive any laser treatment, had a less differentiated epithelium than the groups that received laser therapy. In the group with lower dosage of 1.3 J/cm^2 presented a better fiber pattern than the 3 J/cm^2 group.

Lower dosages seem to give better results but at least a dose with 0.5 J/cm^2 is needed for fibroblast proliferation *in vitro* (Rezende et al, 2007).

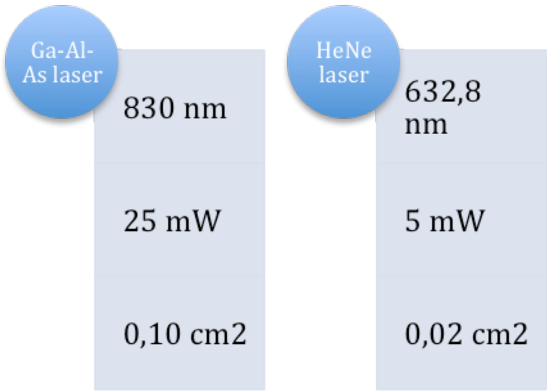
5.4 Oral wounds

Inflammation and conditions in the oral cavity can affect both humans and animals in various regions of the mouth, oral mucosa, gingiva, periodontium, alveolar mucosa, and the mucosa of the tongue and the pharynx. The inflammation can be either primary or secondary and the development of wounds is not uncommon. Several conditions are known in our companion species that will increase the risk of oral wound. These are, among others, different periodontal diseases and feline stomatitis (Riegel & Godbold, 2017).

Oral wounds, or ulcers, can occur on the hard palate due to several different reasons such as trauma, tumor removal or teeth conditions and removals. The last step of the wound healing is the granulation formation mediated by the fibroblasts. They secrete extra-cellular matrix and collagen, which contribute to the closing of the wound. Laser therapy can be a good complement to healing of wounds also in the oral cavity (Fahimipour et al, 2016).

In a study by Fahimipour et al, 2016, mentioned above, the authors made a comparison to investigate the difference between Ga-Al-As and HeNe lasers on oral wounds in both diabetic and non-diabetic mice. The specification of the methods is shown in figure 2 on the next page.

Figure 2. Treatment protocol for the two different laser types in experiments carried out by Fahimipour et al, 2016. Treatment time was 16 sec and dose was 4 J/cm^2 for both groups.



After the intervention the mice were treated with antibiotics to inhibit bacterial growth. The control had a higher level of inflammation than both laser groups after 3 and 7 days, no significant difference could be observed between the laser treated groups. From day 3 to day 7 there was a decrease in the number of inflammatory cells and the inflammation was barely noticed on day 14 in the laser groups.

The HeNe laser had higher efficiency of the two in enhancing the healing processes in the oral wounds, both in diabetic and non-diabetic mice, when based on the overall results. Both types of lasers were more effective regarding the stimulation of fibroblast proliferation and the overall wound healing than the control (Fahimipour et al, 2016).

5.5 Chronic wounds and the JAK/STAT pathway

When a wound fails to go through the normal processes of healing it will develop into a chronic wound. Factors which increase the risk of this development includes raised amount of pro-inflammatory cytokines, reactive oxygen species (ROS), senescent non responsible cells, persistent infection, increased amount of proteases, stem cell dysfunction or stem cell deficiency as well as reduced response to growth factors and cellular signals. Another reason for chronic wounds may be a non-proper function of the cellular cytokine pathways.
One of the most important pathways in wound healing for signaling is the Janus kinas (JAK) signal transducer and the activator of transcription (STAT).

The JAK/ STAT pathway promotes regenerative cell proliferation. When the STAT genes in the body are stimulated it increases the tyrosine phosphorylation in intestine epithelial cells up to 16 times, depending on the STAT gene involved. In diseases where an increased proliferation is not desired, such as neoplastic conditions or immune diseases one of the ways of treatment is to inhibit or reduce the JAK/STAT pathway.

In a chronic wound, the goal is to enhance cell proliferation to increase the healing (Jare et al, 2017)

Laser treatment of both animal and human cell culture has proven to affect the gene expression and be able to induce the production of both growth factors and various cytokines. (Peplow et al, 2011)

When a cell dies, either through trauma or by apoptosis ROS are generated. This leads to the activation of the JAK/STAT signaling pathway and the production of growth factor may be increased, as well as cell proliferation and migration. A similar process seems to take place after laser therapy which might be a good reason for the use of laser therapy also on chronic wounds (Jare et al, 2017).

5.6 Diabetic patients

Wound healing can take longer and shorter time depending on the medical status of the animal. Several studies on wound healing with the help of laser therapy have been made on rats with induced diabetes. Pancreatic insulin insufficiency will lead to high levels of blood glucose and this may lead to retinopathy, nerve damage, stroke and even to limb amputations. The high level of blood glucose in diabetes narrows the vessels when sugar based complexes builds up inside them. This will lead to poor circulation and the nerve functions decreases. In a diabetic patient, when the circulation is disturbed the wound can develop into a chronic one. One of the characteristics of a bad quality wound is a lower value of fibroblasts compared to a normal case and the arrangement of collagen is poor in the proliferation phase (Lau et al, 2015).

5.6.1 Wounds in diabetic patients

A study by Reddy et al, 2001 is dealing with the question if laser therapy can accelerate the wound healing in diabetic rats.

All animals in this study were used as their own control and received two wounds where one was treated with laser therapy and the other served as control.

Laser therapy started the day after the creation of the wounds and the dose used was 1.0 J cm^2 with a laser beam of 632.8 nm. The laser was applied to the wound transcutaneous,

about 5 mm from the skin. Duration of the treatment was 5 days a week until the wound would close, approximate 3 weeks later.
The results were received from the two different wounds and from an intact area of the skin for comparison

Non-wounded tissue showed more than a twofold stronger biomechanical integrity than the parts where the wound had been made three weeks before. When comparing the laser and non-laser treated wounds it was a clear difference between them. The maximum load and stress value on the laser treated wound was increased by 16% compared to the control wound. Maximum strain was increased with 27% for the laser treated wounds compared to the control wounds, to the same level as the intact tissue. When comparing the control wound to the intact tissue, the control wounds strain value was lower than the intact skin.
Elasticity was the same for both laser treated and non-treated wounds. The parameter ”Energy to yield point” increased its absorption energy with 47 % with the laser wound. Overall toughness of the healed wounds was up to 84 % higher in the laser treated wound when compared to the non-treated wound. Total amount of collagen was significantly higher in the laser treated wounds than in the control wounds.
The result of this study indicates that remodeling of collagen is more rapid in laser treated wounds than in non-treated ones (Reddy et al, 2001).

Lau et al, 2015 conducted a study of the effect of laser on wounds in diabetic rats. In most other studies, the focus is on the energy density in J/cm^2. In this study, the aim was to examine results depending on the power output and exposure time.
The animals received a wound on their back and were divided into four groups: one control and three different laser treatment groups. All of the laser treated groups received a constant density of 5 J/cm^2 but the output power was different. Group 1 got 100 mW and the exposure time was 50 seconds which equals 0.1 W/cm2. Group 2 had the power output 200mW and 25 seconds, 0.2 W/cm^2, while group 3 had 300mW and 17 seconds, therefore 0.3 W/cm^2. The laser treatments were given in a non-contact way and once a day until day 9 after the wound making.

Wound contraction rate was increased on day 3 and on day 6 in the irradiated groups compared to the control. The control had 27± 15.7% and 44± 21.1% on the days 3 and 6.

Group 1 had 44± 8.9% and 83± 12.4% on the same days. When group 2 was compared to the control they still had more benefits than the control but not as well as the group 1. In group 3 on the other hand, they showed good progress on day 3 but on day 9, the healing was delayed.

The overall results showed the same tendency in the wound healing for the total 9 days as can be seen in figure 3 (Lau et al, 2015).

Figure 3. Wound healing tendency after 9 days, in percentage of the original wound size, in wounds made on diabetic rats. Control and three groups which recived different output power. The results were with Control ± 31.17%, G1 ± 22.53%, G2; ± 27.98%, G3; ± 26.89% (Lau et al, 2015).

The histological results showed similar tendencies as several other studies, the irradiated groups had better performance. The epithelium close to the wound surface had better proliferation, in the dermis layer the connective tissue was denser than in the control group. In the laser groups, but not in the control group, new blood vessel had been formed. Here as well as in the wound closure rate, group 1 had better results in repairing the damaged area and collagen fibers were seen on day 3. In the control group on the other hand inflammation was still remaining on day 6 and the connective tissue was loose.

At the edges of the wound in group 1 on day 6 there was intense granulation tissue formation and there was only mild or minimal inflammation. In group 2 there was mild inflammation. The group 2 showed more organized and denser connective tissue than the group 3. Upon day 9, both the control and the laser groups had total coverage of the epidermis and stratified keratin was formed at the superficial layer. The difference between the groups was that in group 1 the connective tissue appeared mature and with the irregularity of a normal skin and functional for protection. Also in group 2 it was close to normal with hair follicles even if the connective tissue differed from regular to irregular (Lau et al, 2015).

These studies showed that the healing processes are enhanced and the structure and biomechanical parameters of a wounded tissue in diabetic patients are better when laser treatment is applied than in their controls, which did not receive laser treatment.

6. Microbial growth problems in veterinary medicine

Different medical situations reveal different problems. A problem encountered in orthopedic surgical practice and during maxillofacial surgeries is the chronic osteomyelitis, which can develop. Chronic osteomyelitis is an infection in the cortex of the bones as well as the bone marrow. Most of the time it is *Staphylococcus aureus* which is the causative agent. The main problem with *S. aureus* is that up to 95% of the strains are resistant to penicillin and between 40 and 50% of them have developed resistance against methicillin. This makes the infection hard to treat and with the ischemia and reduced blood circulation in the inflamed area it is hard enough without resistant bacteria (Kaya et al, 2011).

6.1 The effects of laser light on microbial growth

Several microorganisms have been shown to be affected by laser light, both pathogenic and opportunistic species of bacteria and fungi. Studies have been carried out on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and on *Candida albicans.* There is a difference in the microbial growth proved in *in vitro* and *in vivo* studies. In *in vitro* studies there have been both decreased growth, no change on growth and also increased growth. Interestingly, the *in vivo* studies shows decreased growth and this is more or less widely accepted. Several case studies also point in this direction. The norm when using laser therapy for microbial growth in practice is to change, most often to increase, the dosage depending on the evaluation of the result (Riegel & Godbold, 2017).

The type of laser machines with Ga-AI-As diode lasers can penetrate deeper and hence reach the bones with osteomyelitis. Laser with wavelength between 405 and 880 nm have been proven to have bactericidal effect on *S. aureus* (Kaya et al, 2011).
Another effect of how laser therapy can treat infection is by enhancing the functions of the macrophages and modulating the immune response in the body. It can also increase the

production of reactive oxygen species, which then might impact the microorganisms. If used correctly on the treated patient, the laser probe does not induce any thermal pain to the animal and since it increases the endorphin release and has potential analgesic effects, the animals many times like the treatment (Redondo, 2015).

6.2 Laser therapy on bacterial infections in wounds

Kaya et al, 2011 studies the effect of laser in chronic osteomyelitis.

Rats were used for the study where they underwent surgery to expose the tibia and an intramedullary cavity was made. In group 1 no bacteria were introduced, it was used as sham control. The other groups (2-6) were given *Multiresistant Methicillin Staphylococcus aureus* (MRSA) bacteria. The *MRSA* introduced were sensitive to vancomycin but resistant to methicillin. Osteomyelitis was left to develop for 3 weeks after the introduction surgery.

Group 2 were non treated control. Group 3 underwent debridement surgery to clean up the wound but no laser therapy. Group 4 got debridement surgery plus laser 60 seconds with 6 J, 7.64J/cm^2. Group 5 got surgery and twice the time with laser, 120 seconds with 12 J at 15.29 J/cm^2. The last group, group 6, received surgery plus 180 seconds with 18J at 22.93 J/cm^2. The groups which were treated with lase received it directly after the surgery and once each day for 5 days in a row. This is summarized in the table 4 below.

Table 4. Grouping and treatment protocol in the experiments on rats carried out in the study by Kaya et al, 2011.

	Bacteria	Surgery treatment	Dose of laser	Time of laser
Group 1	No bacteria	-	-	-
Group 2	MRSA	No surgery	No laser	-
Group 3	MRSA	Debridement surgery	No laser	-
Group 4	MRSA	Debridement surgery	6 J, 7.64 J/cm2	60 seconds
Group 5	MRSA	Debridement surgery	12 J, 15.29 J/cm2	120 seconds
Group 6	MRSA	Debridement surgery	18 J, 22.93 J/cm2	180 seconds

In the negative control group which did not receive any treatment many inflammatory indications were shown such as dense polymorphonucleated leukocytes, degenerated cells, macrophages, necrotic cells, vascular congestion, apoptotic cells, periosteal inflammation as well as bone necrosis. It was also a low level of plasma, vascular proliferation and lymphocytes. In group 3 with debridement surgery and without laser treatment a decrease could be observed in the number of polymorphonucleated leukocytes and in the vascular congestion. An increase was noted in plasma, macrophage and lymphocyte level when compared to the control. In the irradiated groups of 4, 5 and 6, there was a reverse relationship between the number of lymphocytes and vascular congestion compared to the laser doses. All three of vascular proliferation, fibrous tissue and newly formed bone were observed.

The histological results showed that increased doses of laser, plus the very important debridement surgery, showed a linear relationship with the decrease of the parameters of inflammation which in the end reduced them to match with the levels of the positive control in group 1.

The microbial count had the highest numbers in group 2, with $5x10^5$. Compared to that the reduction rates of group 3 were 26%, group 4 were 54%, group 5 were 70% and a 91% reduction was noted in group 6. The radiographs taken did not show any clear difference between the 21 or 42 days after the introduction surgery.

This study showed that for reduction of MRSA growth in bones, a higher dosage is more beneficial, within the limits between 6 and 18 J (Kaya et al, 2011).

6.3 Microbial growth in vivo

In the article by Redondo, 2015, two case studies are included. The laser machine used both continuous and pulsating light, with 3 different wavelengths: 660, 800 and 970 nm.

The first case is a follow up after an acute trauma surgery. The hip was broken and it had a traumatic abdominal hernia. After the surgery antibiotics were used for 3 weeks: cephalexin, metronidazole and ciprofloxacin as well as NSAID carprofen. The symptoms were pain, lower level of activity, and body weight loss of 30 % in the last 3 weeks, a 12 cm abdominal wound and inflammation. It was not possible to isolate bacteria from the blood. Laser therapy was given for a span of 2 weeks with total 8 sessions. The starting dose chosen was 4 J/cm^2, which was increased over time up to 10 J/cm^2. After one week both the treatments with antibiotics and NSAID could be ended and the dog recovered.

The other case in this article was a wound after an abdominal surgery for removing foreign object in the digestive tract. The dog had been treated with a wide range of antibiotics in the cascade reaction including the following: cefovecin, amoxicillin, ampicillin, cephalexin, gentamicin, doxycycline, ciprofloxacin, enrofloxacin and marbofloxacin. It had multiresistant *Klebsiella pnuemoniae* and probably a secondary infection with *Candida* that was sensitive and responded to amikacin. The starting dose was 4 J/cm^2 and it was increased gradually to 15 J/cm^2 at the end, with 10 sessions in total. After these sessions the wound closed and the dog healed (Redondo, 2015).

6.4 Bacterial growth in vitro

In the study by Nussbaum et al, 2003, they examine the effects of laser irradiation and radiant exposure on different kinds of bacteria *in vitro*, when grown on agar plates. Three different kinds of bacteria were used: *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Different bacteria reacted differently on the wavelengths used and their aim was to investigate this further. When using high irradiance on *S. aureus* with 0,54 W/cm^2 with a He-Ne laser, up to 55% of the bacteria died but the same was not true for the *P. aeruginosa.* These results are an indication that different bacteria react differently to the same exposure. There is a law of photobiology, with the following equation: (Irradiation) x (Time) = (Radiant exposure)

This equation is valid as long as the irradiation or time is not altered with more than a factor of five. Some other studies indicate that this is not the case with bacterial growth as they are rapidly growing and dividing cells, as the irradiation may be more important than the time used. When comparing different use of this equation, it is important to keep other factors, such as incubation time of the bacteria, the same.

During this study the authors used a laser with wavelength of 810 nm with continuous mode. In the first part the radiant doses were control (0) and then 1, 2, 5, 10, 20, 30, 40 and 50 J/cm^2. The irradiation was 0.015 J/cm^2 and only the exposure time was different. In the second part the irradiation was 0.03 J/cm^2 and the radiant were set to control (0), 1, 2, 5, 10, 20, 30, 40, 50, 60, 70 and 80 J/cm^2. So in the second part, the dose rate was double and the time was then half to get these results. Incubation after treatment was 20 hours.
Overall it was shown that the results were very different depending on the species of bacteria.

<u>*E. coli;*</u> The measurement of the cfu (colony forming unit) for *E. coli* was done after treatment with an 810 nm wavelength laser. The irradiant were 0.015 J/cm^2 and only the 30

J/cm^2 showed any decrease of the cfu compared to the control. In the 0.03 J/cm^2 there were more decrease of growth; at 30, 40 and 60 J/cm^2 radiation.

P. aeruginosa; Wavelength of the laser was 810 nm and on the first series of treatments with irradiation of 0.015 J/cm^2 there was a decrease on the number of cfu when the radiation were 5, 10, 20, 30, 40 and 50.

In the second series with the irradiation of 0.03 J/cm^2 there was decrease of the cfu in all the radiations except for 70 J/cm^2 where it was a very slight increase with 1-2% of increased growth compared to the control.

S. aureus; Also here the wavelength was 810 nm and two series was used, the first with 0.015 J/cm^2 irradiation and the other with 0.03 J/cm^2. In the first set the decrease of cfu were on 5, 30, 40 and 50 J/cm^2 radiation. The second set showed decrease in 5, 50, 60, 70 and 80 J/cm^2.

It was concluded that the factor that was of importance was the irradiation energy more than the exposure time (Nussbaum et al, 2003).

When using laser therapy on bacterial growth *in vitro* the results depend a lot on the bacterial species treated and the dosage of the laser treatment. To receive as good results as possible it is important to know what kinds of bacteria the target is and apply the correct dose.

7. Bone repair

The treatment of bone healing and repair with the help of laser therapy is still under research and not as well investigated as in other fields. Some research has been carried out on rats in vivo, and one study covers the use of laser to promote proliferation of human osteoblasts in vitro.

There are many different factors which can impact bone healing such as hormones in the body, the amount of vitamins and minerals available, the local vasculature, weight bearing and exercise, the diet as well as ultrasound and other electrical stimuli. Some studies show that laser treatment enhance the activity level of several cells within the fractured area including red blood cells, fibroblasts, macrophages, osteoclasts and chondrocytes. Some studies show that laser therapy can cause a twofold enhancement of the bone repair after a whole injury in rat tibias. It can also increase the optical density of a bone when comparing laser and non-laser treated groups of animals (Luger et al, 1998).

It is shown that ultrasound waves can positively influence inflammation, the remodeling and repair of bone by enhancing angiogenesis, osteogenic and chondrogenic activity. When laser is applied on bone injuries it can increase the structural stiffness of the callus. By increasing the activity of osteoblasts, and increase the vascularization and the organization of the collagen fibers together with the ATP level rise it can accelerate the bone formation (Lirani-Galvao et al, 2006).

Laser treatment works by induction of the protein mitogen-activated kinase, same as the extracellular signal regulated protein kinases phosphorylation in the myogenic cells. It has also shown to have positive effect of scar tissue formation in animal models. On the cellular level, alkaline phosphatase ALP has been used as markers to follow the route of pre-osteoblasts maturing into osteoblasts. Mature osteoblasts produce extracellular matrix, which builds up the bones with several proteins such as collagen-1, osteocalcin, osteopontin and bone sialprotein (Stein et al, 2005).

7.1 Laser treatment compared to ultrasound in bone repair

One of the bones fractured most often in animals is the tibia. It is associated with delayed and non-uniform healing. In studies both low intensity pulsed ultrasound and low level laser therapy have been used to reduce the incapacity during the healing and the negative side effects during this time.

In the study by Lirani-Galvao et al, 2006, an osteotomy was surgical made at the upper third of the tibia in rats. The rats were divided into 3 groups. The first group received ultrasound with low intensity, pulsating waves. Group two received low level laser therapy with a wavelength of 780 nm, 30mW, 112.5 J/cm^2 in 12 session which were each 2.5 minute. They were given 5 times per week with the start 3 days after the surgery. The laser dosage was chosen to match the ultrasound energy as the aim of this study was to compare the two. The third group was a control group. 20 days after surgery the animals were sacrificed and the results checked. The results are shown in table 5 below.

Table 5. Histomorphic results of the experiment carried out in rats with osteotomy surgery 20 days earlier. The difference in the parameters can be evaluated between the control and the laser treated group (Lirani-Galvao et al, 2006).

Means of the histomorphometric results		
Parameters	Control	Laser therapy
Bone volume %	44.57	47.48
Trabecular separation µm	73.6	63.13
Trabecular number/mm	8.13	9.05
Trabecular thickness mm	54.28	53.76
Eroded surface %	5.37	9.71
Osteoclast surface %	2.19	2.9
Osteoclast number/mm^2	10.61	19.58
Osteoid volume %	1.83	3.09
Osteoid surface %	23.75	37.999
Osteoblast surface %	6.57	11.38
Osteoblast number/mm^2	56.13	117.84

Maximum load at failure showed a significant difference between the laser group two and the control with the numbers 78.1 ± 15.3 compared to 62.5 ± 10.5. The control group had higher bending capacity, which is thought to be because less ossified and healed bone will be more elastic and thus bend more before reaching the breaking bone, as more healed bone will be more static. Regarding the tissue area the groups had more or less similar results. The osteoid volume was higher in the laser group and this group also had higher number of osteoblasts per unit tissue area, both when compared to the ultrasound and when compared to the control. In the other parameters the ultrasound group had higher results or there was no difference between the laser and the control groups.

It can be concluded that the laser therapy stimulated the proliferation of the bone cells and the activity of the ALP that could be seen in the activity of the osteoblasts. It may increase the intracellular calcium levels but it is not clear how this mechanism works. When looking at the percentage comparison it shows that the laser therapy can accelerate the bone repair processes, as the bone formation phase was predominant in the laser groups (Lirani-Galvao et al, 2006).

7.2 Mechanical properties of bone healing

Luger et al, 1998 made a study on rats to examine the mechanical properties of bone healing with the use of laser therapy.

Under anesthesia the right tibia of rats underwent surgery and the tibia was broken and kept back in place with a wire. They were then divided into two groups, laser treatment group and a non-treated control.

Laser irradiation was applied to the laser group at a wavelength of 632.8 nm with 35mW. A HeNe laser probe was held at a distance of 20 cm from the tissue. Sessions were 30 minutes long, given daily for 14 days in a row. The 30 minutes were dived up with 10 minutes each above, under and right on spot of the fracture. In the total the treatment gave 892 J/cm^2 and an energy level between 26 and 52 J/cm^2 as it has been shown by other studies that it is needed for bone repair. The control group got the same surgical treatment but no laser therapy.

The results were presented after 4 weeks, as after 2 weeks the bones were graded as being too immature for the tests and at 6 weeks after the surgery they were already fully united. The mechanical properties were checked after 4 weeks, see table 6 below.

Table 6. Mechanical properties of rat tibias, 4 weeks post breaking surgery. Difference can be evaluated between the control group and the laser treated group that received the treatment with 632.8 nm wavelenght and 35mW (Luger et al, 1998).

The parameters checked at 4 weeks were;

Parameter		Laser	Control
1.	Maximum load at failure (N)	74.4 ±43.1	46.5 ±20.2
2.	Maximum staple area of callus (mm^2)	19.3 ±3.8	22.9 ±5.7
3.	Stress high load (N/mm^2)	3.78 ±2.48	2.18 ±1.23
4.	Extension maximum load (cm)	2.64 ±1.15	4.43± 2.90
5.	Structural stiffness of tibia, callus stiffness (N/mm)	26.28± 11.88	13.97 ±10.27

The parameters ”Maximum load at failure” ”Stress high load” and ”Structural stiffness of tibia” were all significantly higher in the laser treated group than in the control group. On the contrary the ”Extension maximum load” was closer to twice as high in the control group as in the laser treatment group. In the last parameter ”maximum staple area of callus” there was no large difference. In the control group the calluses appeared to be more fibrocartilaginous and with less ossification and the laser treatment group had already started to show more of a bony structure. The authors think these results may depend on the callus in the control group that was larger and not as strong as in the laser treated group.

Interestingly, in the control group 21% of the rats had non unified bones 4 weeks after surgery, while the number was 0% in the laser treated group. After all, the important part of a bone healing process is the end result where this study shows that low power laser clearly enhances the healing of bone, at least in rats (Luger et al, 1998).

7.3 Laser treatment on osteoblasts *in vitro*

This study by Stein et al, 2005 focus on the proliferation of osteoblasts derived from human bone. The cells were cultured on special medium and exposed to laser irradiation after 24 and 48 hours post inoculation. The laser used was a HeNe laser with a wavelength of 632.8 nm, power were 10mW and the power density 180 mW/cm^2. Different groups of culture received laser treatment for different time periods of 1, 3 and 10 seconds which gave the energy density of 0.14, 0.43 and 1.43 J/cm^2. For the control normal room light were used for the same amount of time and they were also out of the incubator the same amount of time as the irradiated cultures. The result parameters were the number of cells, the alkaline phosphatase (ALP) activity, the osteopontin and the bone sialoprotein.

In the group with 3 seconds treatment there was a significant increase in the number of cells compared to the control group, about 25% more on both the 24 and 48 hour check after the second treatment. In optical density another significant increase was observed in the group with 3 seconds treatment with 40% and 38% at 24 and 48 hours after the second treatment compared to the control group.

Regarding the ALP activity it was an increase in the 1 and 3 second treated groups compared to the control group. The tendency was the same at 48 hours post the second treatment. In the cultures being exposed for 10 second and thus 1.43 J/cm^2 this tendency was not as significant as they showed only a slight increase. The pattern for ALP activity occurred both for the osteopontin and the bone sialprotein. They were higher after the 3 seconds laser treatment compared to the control (Stein et al, 2005).

This study clearly showed that a short laser treatment can positively influence the proliferation of human osteoblasts when grown *in vitro*. It also increases the differentiation, which may lead to better healing if applied *in vivo.*

8. Inflammation

The inflammation reaction is very similar to the process after an acute injury with many of the cells and factors being involved in both conditions. This is explained above under the chapter 5, Wound healing and tissue repair.

In the inflammatory reaction in the body the prostaglandins play an important role. Their presence impacts the inflammation in the tissues and by affecting them and other factors it is possible to alter the level of inflammation.

After laser treatment both the synthesis and the secretion of prostaglandins may be inhibited. On the contrary to inhibition, growth factors and cytokines are stimulated from laser therapy. They have antioxidative and anti-inflammatory actions which help reduce inflammation and the sensation of pain. Also cyclooxygenase 2 (COX-2) production decreases and so does the bradykinin level. Bradykinin is involved in long term or chronic pain, it stimulates nociceptive afferent nerves in the skin and the viscera. Laser therapy also reduce the production of interleukin which has a role in the inflammation system. As the circulation is improved with the help of laser therapy, the tissue also gets more oxygen and rest products can be transported away, which may help the sensation of stiffness and pain. Both beta-endorphins and serotonin have been showed to be released during laser therapy which further increases the pain reduction (Riegel & Godbold, 2017).

After the start of acute inflammation several mediators are released by the body, among them prostaglandins, interleukins, leukotrines, NO and ROS. Alteration of these may change the amount of inflammation and thus the amount of edema in the inflamed area (Albertini et al, 2007).

8.1 COX 1 and COX 2

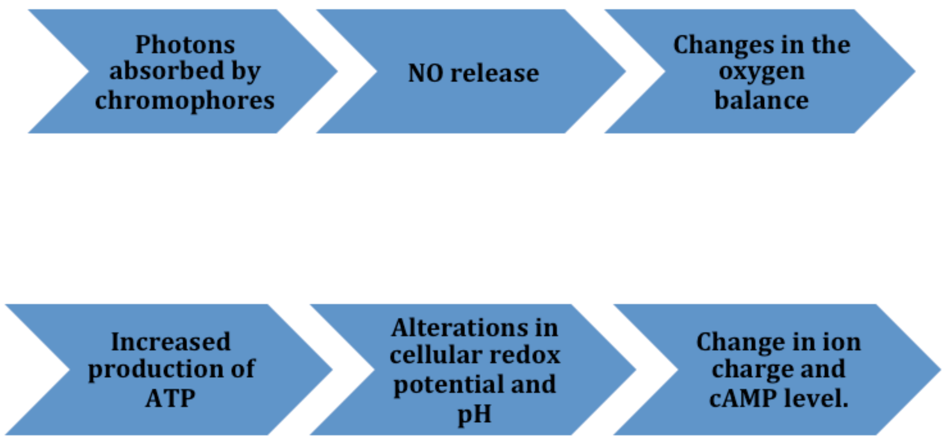
The enzyme cyclooxygenase consists of two different isoforms and are named COX-1 and COX-2. COX-1 is found in most tissues in the body and its main function is to produce precursors for example prostaglandins, among others. One of the important places for COX-1 is in the platelets, where it regulates the aggregation in the blood coagulation cascade. It also plays an important role in maintaining the function of the glandular system in the stomach.

The isoform COX-2 is an enzyme acting in inflammatory conditions, promoting the prostanoid synthesis. Elevation of COX- 2 gene expression elevates the inflammation level of a tissue.

In general this means when the aim is to alter the inflammation, the target is the COX-2 and not the COX-1. The medicine group of Non-Steroidal Anti-inflammatory Drugs (NSAID) is used to alter the expression of cyclooxygenase in the inflammatory process (Dubois et al, 1998).
With treatments against inflammation the target is to decrease the gene expression of COX-2, preferably while non altering or increasing the expression for COX-1 genes (Marcos et al, 2011).

Laser light work by emitting photons which are absorbed by the cyclooxygenase. This leads to the reaction seen in figure 4.

Figure 4. The figure below shows the reaction that takes place in the cell when it receives laser light and explains how the stimulation takes place. 1. Photons in the light are absorbed by the chromophores in the cell, one of the most common being cytochrome C. 2. Laser light also leads to the release of N0, 3. This leads to changes in the respiratory chain and the activation of the enzyme flavomononucleotide. 4. The production of ATP increases and the metabolism of the cell is changed (Millis & Levine, 2014). 5. When the level of ATP is increased the cells redox potential and the pH is altered, this causes 6. A change in the ion charge and the cAMP level More studies are needed to fully understand the complete cellular function (Jare et al, 2017).



When these factors activate the signaling pathways it will lead to an increase of the proliferation and migration of the cells. The production and secretion of both growth factors and cytokines will be increased (Jare et al, 2017).

Marcos et al, 2011 presented a study where laser treatment were compared to the NSAID diclofenac. Diclofenac is a common drug used to decrease inflammation.
In this study tendonitis were induced in rat models by injections of collagenase. They were treated either with diclofenac injections or laser therapy. The treatment protocol for the laser was 1, 3 and 6 J applied 1 hour after the tendonitis induction. The wavelength was 810 nm, output was 100mW and the times were 10, 30 and 60 seconds, corresponding with the raised dosages of joule. One group served as control and received no treatment for the tendonitis.

Results were evaluated 2 hours after the collagenase injection. Regarding COX-1 there was no difference between either of the laser groups or the diclofenac group but they all showed an increased expression compared to the control group.

For COX-2 the results were more divided. The lowest laser group with 1 J showed no difference to the diclofenac group and the gene expression was not significantly altered compared to the collagenase control. Both the other laser groups showed more promising results. The 6 J dose group made a significant decrease of the COX-2 compared to the control group, and the 3 J dose group had a highly significant decrease. The decrease of the 3 J group was lower than the saline control, with no induced tendonitis (Marcos et al, 2011).

This shows clear evidence of the effect of laser therapy on tendonitis in rats and points towards the use of laser therapy in everyday veterinary medicine practice.

8.2 Edema formation

When tissues get inflamed the amount of fluid in them increase and they become edematous. In an inflamed tendon the wet and dry weight differs from a healthy tendon and this can be used as an indication of the level of inflammation.
In a trial different groups of rats with induced tendonitis received either NSAID or laser treatment with 1, 3 or 6 J dosage and a control group. When comparing the four treated groups no main change could be seen in the NSAID, 1 J or 6 J laser treated groups when compared to the control tendonitis group. The dose of 3 J showed larger change and the edema was significantly reduced in this group compared to the control groups when applied on rats with induced tendonitis (Marcos et al, 2011).

Albertini et al, 2007, carried out a study on the edema formation in rat paws after induced inflammation. The rats were divided into 4 groups. One control group without inflammation, another control group with inflammation and two laser treated groups with inflammation. Both laser treated groups received a dosage of 7.5 J/cm^2 and the output was 30 mW. The difference between the laser treated groups was the wavelength. In the first group it was 660 nm and in the other it was 684 nm. The laser treatments were carried out once, 1 hour after the induction of the inflammation.

Three hours after the induction of inflammation both laser therapy groups showed a significant decrease of edema compared to the inflammation control. When the amount of edema was checked 4 hours after induction the laser treated groups had 0.58 ±0.17 ml for the 660 nm group and 0.76 ± 0.1 ml for the 684 nm group. The inflammatory control had 1.67± 0.19 ml. In percentage, the reduction was 65.3% in the 660 nm laser group and 54.5% for the 684 nm laser group, when compared to the inflammatory control.

Regarding the cellular results, the non-laser treated inflammation group showed regular signs of inflammation with an increased number of inflammatory neutrophil cells while both the laser groups had close to normal features with just small signs of inflammation.

The overall results showed that there was a similar response in the inflammatory processes of both groups treated with laser regardless the wavelengths. The results were better in the laser treated groups than the inflammatory control (Albertini et al, 2007).

8.3 Matrix metalloproteinase

Matrix metalloproteinases (MMP) are proteinases belonging to the family of metzincin. Their function in the body includes the maintenance and remodeling of tissues. They can degrade extracellular matrix and regulate other tissue functions by the secretion and activation of cytokines, chemokines and growth factors. (Löffek et al, 2011).

MMPs are important in the morphology of the tendons as they are able to degrade collagen and proteoglycans.

Traditional treatment of inflammation is the use of different drugs in the NSAID category. Lately the suspicion has been raised that they might be harmful to the tendons by the interference with the MMPs. This increases the demand for alternative treatment method, where laser therapy might be one (Marcos et al, 2012).

Marcos et al, 2012 made a study of how the NSAID diclofenac compared to laser therapy can influence the biomechanical properties of the Achilles tendon in rats.
Wavelength were 810 nm, power density 3.56 W/cm^2 and the groups had doses of 1 respective 3 J. Laser treatment was given once at 1 hour after tendinitis was induced with a collagenase injection. The evaluation took place 2 hours post induction of tendonitis on three different metalloproteinases; MMP-3, MMP-9 and MMP-13.

For the MMP-3 gene expression neither diclofenac injection nor the laser treatment with 1 J could show any reduction compared to the non-treated control group. The 3 J group on the other hand, showed a significant reduction of the gene expression 2 hours after the induction of tendonitis.
The results for MMP-9 showed that the diclofenac treatment increased the gene expression while both the 1 and 3 J laser groups had a decrease, all compared to the control group, with the 3 J dose group showed slightly better results. The tendency was the same for the gene expression of MMP-13.

Gene expression of MMPs can change with both drugs and laser therapy. Different dosages of laser can affect different gene expressions and more studies are needed to fully understand how laser treatment can be a good complement to medication (Marcos et al, 2012).

8.4 TNF- alpha

Tumor necrosis factors alpha (TNF-**α**) is a pro-inflammatory cytokines which can stimulate the synthesis of MMPs. In a study with induced inflammation in rat tendons, the amount of TNF-**α** was highly increased compare to normal tendons without inflammation. When laser dosages of either 1 or 3 J were applied there was a significant decrease in the gene expression of TNF-**α**. This decrease was not shown for the rats receiving diclofenac injections instead of laser treatment (Marcos et al, 2012).

9. Pain reduction

Laser therapy seems to reduce the sensation of pain, not only by the decrease of the inflammation. Several theories exist for the mechanism behind this, for example;

- local vasodilatation
- increased microcirculation
- increase of the nociceptive threshold
- inhibiting neuronal effects on peripheral nerves
- increase endorphin release

It is harder to measure pain in animals than in human patients, so more studies have been carried out in this field regarding humans than animal models (Riegel & Godbold, 2017).

Jang & Lee, 2012, made a meta-analysis of 22 trials with human patients treated with laser therapy for pain in joint areas. Both studies using laser therapy alone and in combination with drugs or exercise were evaluated. They concluded that laser treatment was a good complement to painful joint conditions in humans when the correct dose for the specific area was used, as different areas needed different dosages. They stated that laser therapy has no reported side effects which make it a good alternative to treatment with NSAIDs (Jang & Lee, 2012).

Another meta-analysis study showed that in overall laser therapy is highly effective for pain control (Enwemeka et al, 2004).

In a study by Goats et al, 1996, the human patients in the trial showed a significant increased passive range of movement (PROM) in several joint groups for the laser treated group compared to the placebo control. This indicates a decrease of pain in the joint affected (Goats et al, 1996).

Bjordal et al, 2003, made a large systematic review of laser treatment on painful joint disorders. They concluded that laser therapy showed to be effective in the reduction of pain in different joint conditions in humans by their way of decreasing the inflammation (Bjordal et al, 2003).

9.1 Painful knee osteoarthritis

A study carried out by Hegedüs et al, 2009, in human patients with knee osteoarthritis had a laser treated group and a placebo group. The laser group receives treatment with a Ga-Al-As diode laser with the wavelength 830 nm, power 50mW and the dose of 6J/point which gave a total of 48J/cm^2. The placebo group was treated with the same type of device but with a non-efficient probe.

Treatment took place two times a week for 4 weeks, in total 8 sessions. For evaluation the parameters used were a pain scale, pressure sensitivity, flexion degree and circumference in centimeters.

Joint flexion in the laser treated group showed a significant increase which could be seen between each measurement while the placebo group did not show this trend at all.

For pressure sensitivity the only change was between first and last measurement in the laser treated group with a significant decrease in sensitivity. For the placebo group this was not noted. Concerning the pain relief the laser treated group showed a significant decrease in pain at each measurement while this was not the case for the placebo group. The circumference of the joint showed no significant change in either group.

Two months after the treatments the laser treated group still experienced less pain compared to the placebo control. This shows that laser treatment is a relevant treatment method and may work long term even after the end of the treatment sessions (Hegedüs et al, 2009).

9.2 Laser therapy combined with exercise

In a trial with human patients the question was if laser therapy in combination with exercise could be more beneficial than exercise alone when dealing with chronic lower back pain. The laser type was a Ga-Al-As diode with wavelength 810 nm, 50 mW output and dose 27 J/cm^2. Laser treatment was given twice a week, 6 weeks in a row and the results were recorded at week 6 and week 12. Pain was evaluated with a questionnaire and a pain scale.

At the 6 week checkup, there was a decrease of 95% in the combined group with both exercise and laser therapy, this trend was not observed in any other treatment group. It was concluded that the laser therapy helped not only to decrease pain but increase the lumbar flexion and to reduce the disability, if used together with exercise (Djavid et al, 2007).

9.3 Pain reduction in rats

Chronic pain, especially in the lower limbs is a common symptom in medicine. It may be due to arthritis, tendinitis, stress fractures, facial lesions or different nerve compressions. This can lead to weakness or atrophy of the muscles and reduce quality of life for the patient (Bertolini et al, 2011).

The authors mentioned above, Bertolini et al, 2011, carried out a study regarding the effect of laser treatment on pain in rats. The sciatic nerve was compressed with a ligature during surgery. Three groups underwent the sciatica surgery and laser treatment but the first group, the control, got treatment with the laser probe turned of. The second group received a dose with 4J/cm^2 and the third group a dose of 8 J/cm^2. The wavelength was 830 nm and the power output was 30mW. Three days after the sciatic surgery the laser treatment started and repeated daily for five days. The pain was noted using a standardized protocol.

In the control group, the pain showed increased significant from before and after the surgery and there was a reduction until the 5th day of control treatment. Both the laser group also showed increased pain after the surgery compared to pre surgery measurements. The interesting part was that both the 4 J/cm^2 and the 8 J/cm^2 groups showed no significant difference after the end of the laser treatments when compared to before the sciatic surgery. They were back to normal values, similar to that of a non-interfered nerve. Laser treatment with this parameters clearly made a large change to the pain level in the rats even that more studies are needed to fully understand the mechanisms (Bertolini et al, 2011).

9.4 Different wavelengths used for pain relief

A study made by Masoumipoor et al, 2014 investigated the differences of pain relief with different wavelengths of lasers. Two wavelengths were used in the experiment, 660 and 980 nm. Three groups of rats were created, one control and two laser treated groups. All rats were subjected to a surgery that constricted the sciatic nerve that causes chronic pain. In the control the rats did not receive any laser treatment. The first laser therapy group was treated with a 660 nm wavelength laser with power 100 mW, energy density 4 J/cm^2 and power density 0.354 W/cm^2. In the second group the wavelength was 980 nm, power of 70 mW, energy density 4 J/cm^2 and the power density was 0.248 W/cm^2. Time of treatment was 11.3 and 16.13 seconds respectively. Laser therapy sessions started the first day after the surgery and continued every day for two weeks for both the laser treated groups.

For the evaluation two parameters were used, thermal and mechanical withdrawal threshold. Thermal threshold was measured the time in seconds until withdrawal of the hind paws when a beam of focused radiant heat was applied on the plantar part of the paws.

The mechanical withdrawal threshold was investigated using a machine that pressed the paws under a constant speed and the force was measured in grams before the rats withdraw their paws.

After 7 and after 14 days these parameters were tested and the results can be seen in the table 7 and 8 below.

Table 7. Results of 660 and 980 nm laser treatment on mechanical withdrawal threshold in rats in the experiment by Masoumipoor et al, 2014. The difference can be seen on the mechanical pain threshold in grams before withdrawal on the 7th and 14th day after the pain induction surgery.

	7th day	14th day
Control without surgery	19.18 ± 4.66 g	19.18±4.66 g
Control with surgery	10.17±4.18 g	9.15±4.20 g
Surgery and 660 nm laser	15.54±4.50 g	14.36±5.43 g
Surgery and 980 nm laser	14.15±4.25 g	12.35±5.28 g

Table 8. Results of 660 and 980 nm laser treatment on thermal withdrawal threshold in rats in the experiment by Masoumipoor et al, 2014. The difference can be seen on the thermal pain threshold in seconds before withdrawal on the 7th and 14th day after the pain induction surgery.

	7th day	14th day
Control without surgery	18.91±4.08 s	18.91±4.08 s
Control with surgery	12.42±4.82 s	10.70±5.02 s
Surgery and 660 nm laser	18.34±4.29 s	19.88±3.13 s
Surgery and 980 nm laser	16.13±4.11 s	14.18±3.35 s

According to the results of this study, there were significant differences of both laser treated groups compared to the control group that also received the surgery. Between the different laser treated groups, there was a difference but it was not enough to be classified as significant but the 660 nm wavelength laser treatment seemed to be a bit more effective than the 980 nm treatment (Masoumipoor et al, 2014).

Many factors influence the results of a laser treatment and to fully make a fair comparison, all other parameters should be the same. More research is needed to fully understand the differences and the possibilities when using different wavelengths (Riegel & Godbold, 2017)

10. Conclusions and discussion

The aim of this literature review was to understand the function of the laser treatment on the tissue and to gain knowledge on why and how it works on living animals. Since the field of laser therapy treatment is developing very fast, several new studies came out during this work. More information becomes available all the time about the use of laser therapy in companion animals.

For the laser therapy treatment of wounds, the factor of largest importance was the irradiation dose, measured in joules. In the studies with several dose groups, the lowest dose group was often the group where the results were the best. This was true down to a limit, where the energy was no longer enough to stimulate the cells and hence no improved results were seen. Some changes can be seen depending on the type of laser machine used, but most probably it has more to do with the penetration depth than with the actual medium of the diode. Some attention could also be paid to the wavelengths of the laser used, as one study presented different results depending on the wavelengths used, independently of the dose.
Several studies were carried out on animals with induced diabetes mellitus. This is a disease that severely impacts the quality of the healing processes and the studies showed that laser treatment enhanced the healing in these conditions as well. This can be of importance both in animal and human medicine to reduce unnecessary suffering during a recovery.

Treatment with laser on non-healing wounds, for example after surgery, to inhibit the growth of bacteria is another area which could be applied more if there was more studies carried out to relay on. Since the findings *in vitro* differs from the *in vivo* results the focus needs to be put on carrying out more studies on living animals and maybe even under field conditions, out on real cases to find out if the treatment method can be of real use in the everyday medicine. More research regarding the doses *in vivo* would be of great use as well.

When applying laser on the cellular level there were changes seen both in the signaling pathways and in the gene expressions. This has been researched mostly in the field of inflammation and pain control. Since the stimulation of the cells can change the gene expression for the inflammatory mediators it can be a huge step forward for the treatment of pain and inflammation both in animals and humans but more research is needed to know which doses are the optimal for the different species and the different areas.

The research in the signaling pathways is still under development and it will be interesting to see what is reveled in this field during the next few years.

Even if is not the easiest of tasks to decide the pain level on animals there are many helpful tools being developed such as pain scales and pain face models. Since it is proven that laser therapy can decrease inflammatory conditions in several ways, the step is not far away to use it primarily for pain as well. With the help of the new tools mentioned it should be possible to carry out studies on companion animals and more research is definitely needed in regards to this.

During the research for this literature review I could not find any article stating that laser therapy worsened any condition it was applied on. The only important thing mentioned was that since the laser therapy stimulates the cells, caution needs to be taken when an animal is suspected to have for example neoplasms, where the stimulation of proliferation of the cells is highly undesired. Some authors mentioned that previous studies by others had shown inconclusive or no change of results and when these experiments were emulated under more organized conditions, the results turned out differently. No side effects of laser treatment were noted in any of the studies, which indicate that laser therapy is a safe method and can be applied as a complement to other methods such as medications or surgery.

The conclusion from this work is that laser therapy is being investigated, and used, for a wide range of conditions and it is not without good cause. There is a known, but not fully understood, reason behind the use and the application can be safely carried out when there is common sense involved to take the precautions needed and to avoid treating conditions that should not be stimulated. Laser therapy is not any kind of magic, but a well worth complement to wound and bone healing as well as to inflammatory and painful conditions.

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