Efficacy of low level laser treatment in the management of chronic wounds

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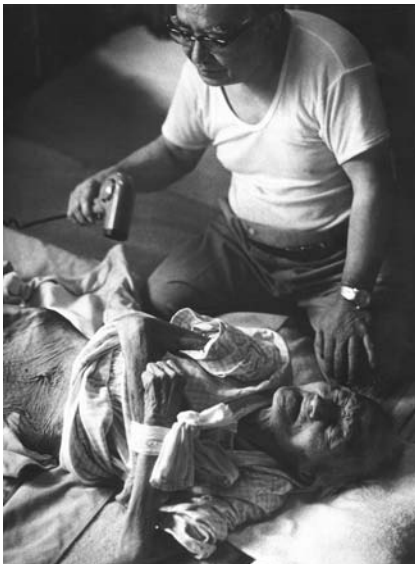

Cees Lucas

Cover: *A 62 year old Japanese man takes care of his 91 year old mother. Over the entire body she suffers from decubitus ulcers, which he treats with a hair dryer. This takes three hours. Her hands are tied, preventing her from scratching the painful areas.*

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Efficacy of Low Level Laser Treatment in the Management of Chronic Wounds

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Voor Roderick en Christopher

Het kan nog directer
correcter
perfecter
het kan altijd
doorspekter
met kwaliteit
het kan nog gesmeerder
georganiseerder
gedisciplineerder
met nog meer beleid

Rob en Martin, bedankt !

Contents

Chapter 1

Introduction

A decubitus ulcer (pressure ulcer, or pressure sore), as the prime example of a chronic wound, is defined as 'any degenerative change, caused under the influence of pressure and shear forces acting upon biological tissues'. [1] The term decubitus is derived from the Latin phrase 'gangrena per decubitum', meaning: 'tissue necrosis, resulting from laying down'.

Many compounding factors contribute to decubitus ulcer formation, but the primary causes are pressure, which compress the vasculature, and shearing and friction forces, which distort and thereby occlude the capillary network and deeper vascular system. [2] Usually these forces combine to some degree and, as a result, decubitus ulcers develop over time. Prolonged occlusion severly limits blood flow, cells are traumatized from resultant ischaemia, and (irreversible) tissue necrosis occurs. [3] Decubitus ulcers are usually located over bony prominences exposed to (external) mechanical compression. Other compounding (internal) factors include, but are not limited to, metabolic disorders, decreased immunity, nutritional deficiencies, immobility, and excessive moisture. These risk factors increase the vulnerability of tissues to destruction from ischaemia caused by prolonged external forces on the skin [4, 5] (Table 1).

Table 1

Pathophysiological, clinical, and patient related risk factors for developing decubitus ulcers
1. Pathologic mechanical and physical states (pressure, shear, friction, moisture, temperature)
2. Skin characteristics
3. Medical diagnosis and physical condition (hypotension, low body weight, dehydration, obesity, cardiac failure, chronic illness, diabetes, contracture)
4. Medication
5. Malnutrition
6. Incontinence
7. Sensory impairment
8. Cognitive deficits
9. Immobility
10. Inactivity
11. Advancing age

In the recent past, a number of classification systems have been defined to describe the visually observable changes in the skin and destruction of the skin. In the United States, the National Pressure Ulcer Advisory Panel (NPUAP) reached consensus by combining several

of the most commonly used staging systems in order to achieve a universally accepted four stage classification system [1] (Table 2).

Table 2

Decubitus ulcer classification system [1], adopted by NPUAP *	
Stage I	Non-blanchable hyperaemia of intact skin, the heralding lesion of skin ulceration.
Stage II	Partial-thickness skin loss involving epidermis or dermis or both. The ulcer is superficial and presents clinically as an abrasion, blister, or shallow crater.
Stage III	Full-thickness skin loss involving damage or necrosis of subcutaneous tissue, which may extend down to, but not through, underlying fascia. The ulcer presents clinically as a deep crater with or without undermining of adjacent tissue.
Stage IV	Full-thickness skin loss with extensive destruction, tissue necrosis or damage to muscle, bone, or supporting structures (e.g., tendon, joint, capsule).
	* **NPUAP:** National Pressure Ulcer Advisory Panel

Examples of these stages are presented in Figures 1-6. The clinical characteristics of the various decubitus ulcer stages, including the pre-decubitus phase can be described as follows:

In the ***pre-decubitus phase*** an initial effect of local pressure application can be observed after pressure relief. The occurring local redness disappears by compressing it ('blanching' hyperaemia). At this phase, there is already slight damage to the blood vessels.

The decubitus process without complications *(Stage I, Figure 1).* If pressure on the tissues continues, the local aseptic inflammatory reaction causes a peripheral redness which also disappears under pressure. In contrast to the pre-decubitus phase, however, the central red area does ***not*** disappear because of bleeding and thrombus formation.
This so-called 'non-blanching' hyperaemia is considered to be the first stage of the decubitus process. At this stage extensive subcutaneous tissue damage may already have developed. This damage is palpable as a solid-elastic subcutaneous tumour. Prolonged pressure on the skin turns the non-blanching redness into a clearly defined blueish-red area, since the subcutaneous necrotic tissue shows through the skin.

Epidermal and, or dermal complications *(Stage II, Figure 2).* In the non-blanching hyperaemia and edema stage, an eczematic skin reaction may occur. This sensitizes the skin to frictional forces, especially if there is moisture between the skin and immediate underlayer (sheet, clothing). Papulae, vesiculae, or bullae develop and, if the skin remains unprotected at this stage, the surface of an occurring blister will be broken and a superficial ulcer is now present.

Subcutaneous complications *(Stage III, Figure 3).* Bacteria will erupt and thrive on the subcutaneous necrotic tissue, causing septic inflammation and more tissue damage. Continuation of pressure causes a black, dry necrotic scab to form, still surrounded by a hyperaemic zone. The ulcer now extends to layers of fat and muscle tissue. If the necrotic tissue is removed, a deeper decubitus lesion with inflammation in the surrounding tissues becomes apparent. At this third stage, the peripheral borders of the ulcer are barely or not at all undermined.

Complications in the deeper layers of the tissue *(Stage IV, Figure 4).* Necrotic tissue often dissolves, leaving a cavity in which bacteria can thrive further, causing abscesses. Phlegmoneous inflammation of the subcutaneous tissues may also complicate a previously isolated necrosis and destroy a considerable area of tissues. When an abscess bursts through the skin, a sinus-shaped decubitus lesion appears. The relatively small superficial skin lesion may often access to an extensive necrotic subcutaneous area (Figures 5 and 6). Under this necrotic tissue layer, there is usually a thick layer of fibrous tissue with clear signs of fibroid degeneration. When abscesses are deeper, there is a risk of fistula formation.

Decubitus ulcers represent a significant percentage of chronic wounds. In the United States, annually 1.7 million patients develop a decubitus ulcer. [6] In skilled care facilities and nursing homes, the prevalence ranged from 2.4 to 23 percent, [7-11] versus 66 percent among elderly patients admitted for femoral fracture. [12] Prevention and treatment of decubitus ulcers is extremely expensive and with respect to the rapid increase of the ageing population in Western Europe and the USA is a matter of great importance. In the United States, the treatment of decubitus ulcers has been estimated to cost $ 6.4 billion in 1994 and $ 8.5 billion in 1997 [13], which is more than the cost of treating patients with AIDS and almost half the amount spent on caring for patients with dementia. [1, 14] In the Netherlands, the direct medical cost of decubitus ulcer prevention and treatment is estimated at $ 700 million annually (1991). [15] In 1999, these figures were confirmed by the Health Council of the Netherlands, ranking the cost of decubitus a third place immediatly after the cost for cancer, and heart and vascular diseases, being 1.3% of the total cost of health care in 1998. [16]

Despite major improvements in health care in general, and the recognition of risk factors for developing decubitus ulcers in particular, a gold standard for decubitus ulcer treatment is currently lacking. This is reflected by the broad range of products and interventions for treating these ulcers and by the absence of a superior treatment with a clearly demonstrated effectiveness in the database of the Cochrane 'Wound Field' and 'Rehabilitation & Related Therapies Field'. The 'Consensus Decubitus' is only considered a guideline with instructions for prevention (e.g. recognition of at-risk patients, special mattresses, frequent alteration of patients' positions), diagnosis (e.g. the use of a descriptive classification system), and treatment (e.g. correction of anaemia and malnutrition, occlusive dressings, antibiotics, excision of necrotic tissue). [1, 6, 13, 14]

Low Level Laser Therapy

Low Level Laser Therapy (LLLT), or 'laser photobiostimulation,' has frequently been suggested as a promising treatment option for open wounds. The Hungarian surgeon Endre Mester was the first to document the biologic effects of LLLT on wound healing in case reports.[17, 18] A number of possible mechanisms of action involved in LLLT has been postulated. With regard to the wound healing process, this so-called laser-catalized reaction includes: stimulation of resorption and diffusion [19], activation of the immune system [20], acceleration of the inflammatory phase of wound healing [20], enhanced prostaglandin concentration [21], ATP synthesis [22, 23,] collagen synthesis [19, 23,] fibroblast proliferation [19, 23,] and phagocytosis of macrophages. [24]

The use of LLLT has been advocated as a primary indication for the treatment in the presence of compromized or delayed wound healing, since. [23] However, the introduction of LLLT has always been surrounded by controversy [25, 26] and the earliest reports of clinical success with this modality were met with scepticism. In contrast, a recent literature review concludes that 'this type of phototherapy should be considered a valuable (adjuvant) treatment for selected therapy-refractory conditions such as the impairment of wound healing.' [27] But even to date, there are medical scientists and clinical epidemiologists questioning the efficacy of LLLT, categorizing this treatment as 'a fringe medical technique for which there is no convincing scientific evidence.' [28] Consequently, the US Food and Drug Administration (FDA) has limited the use of low-energy lasers to 'approved experimental use' [29] and LLLT has yet to receive FDA approval for any indication. [30-31]

Outline of the thesis

The objective of this thesis is to assess the efficacy of Low Level Laser Therapy as a treatment option for stage III decubitus ulcers. Wounds were limited to stage III decubitus ulcers, because these ulcers are well measurable and, in comparison to stage IV ulcers, the laser light penetrates easily in the wound surface. In ***Chapter 2***, we analyzed the results of a systematic review describing the efficacy of LLLT on wound healing in human subjects. In this review the results of four randomized clinical trials, investigating the effects of LLLT versus placebo or any other intervention, are described. For three of these studies we could perform a meta-analysis. In this analysis, we calculated the pooled relative risk increase for wound closure.

A pilot study to investigate the effect(s) of LLLT on stage III decubitus ulcers and the feasibility of a multicenter trial in nursing homes is described in ***Chapter 3***. In this study we particularly focussed on the applicability of our wound registration methods and the extent of wound size reduction. The latter outcome measurement was used to perform a power analysis in preparation of a full scale randomized clinical trial.

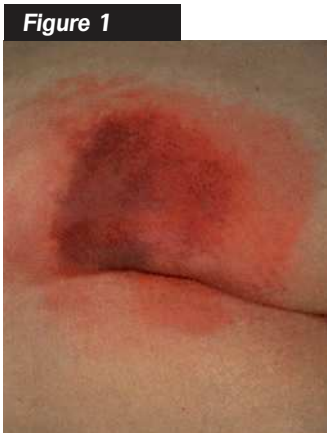
Subsequently, in ***Chapter 4***, we assessed the reliability of our wound surface area measurement method. Since periodic assessment of wound healing is an essential element in pressure ulcer management we investigated the intra- and interobserver reliability of an instant full scale photographic technique, combined with transparency tracing. Using this combined method we intended to reduce the limitations and disadvantages inherent in both separate techniques.

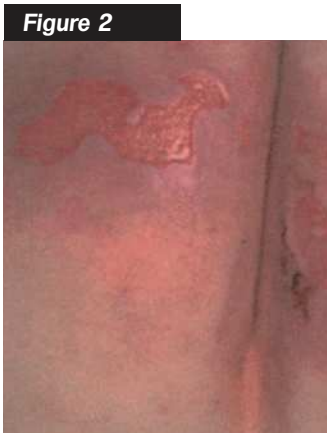
In view of the absence of randomized studies with sufficient large sample sizes in human subjects and based on the results of the previous chapters, we performed a prospective, observer blinded, multicenter randomized clinical trial to assess the efficacy of LLLT in the treatment of decubitus ulcers ***(Chapter 5)***. All patients received the prevailing consensus decubitus ulcer treatment, whereas the experimental group had LLLT as an adjuvant. Of 105 eligible patients, 19 had to be excluded before randomization. Consequently, our results are based on 86 patients with stage III decubitus ulcers.

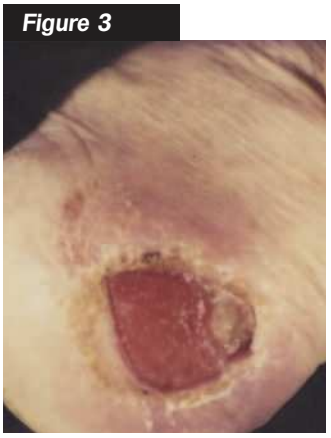
However, as time elapsed, we found that the results from both our systematic review and the randomized clinical trial did not support the hypothesis that LLLT had a beneficial effect in terms of wound closure in stage III decubitus ulcers. This led us back to the foundations of the clinical trials with LLLT; the cell studies and animal model experiments in wound healing. ***Chapter 6*** contains a systematic review of 36 studies, investigating the effects of LLLT in cell studies and animal experiments. This study focussed on the question whether the evidence from cell studies and animal experiments were unequivocally in favour of LLLT, which would imply that these models might be adequate to predict treatment response in patients, or that the data of cell studies and animal experiments were inconclusive, which would mean that the clinical trials were based on insufficient evidence. For 11 of these studies we were able to calculate the pooled effect sizes on a total of 22 outcome parameters. In-depth analyses were performed on five subgroups: [1] studies with primary outcome measures on dimensions with direct reference to wound healing (ranging from acceleration of wound closure to epithelialization, but excluding surrogate dimensions with regard to wound healing; in this case: tensile strenght); [2] studies in which inflicted wounds on animals were irradiated and evaluated; [3] animal studies with 'true controls'; [4] studies in which animals functioned as their 'own controls'; and [5] studies with the highest methodological quality score.

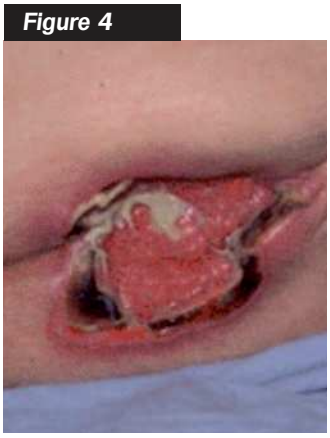
Chapter 7 presents a general discussion and conclusions of our studies and indicates directions for further research. In this chapter, we particularly focus on the theoretical and biological assumptions of low level laser therapy in wound management.

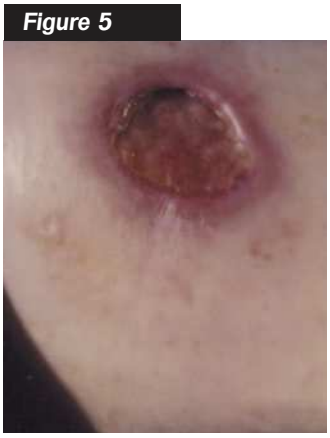
A ***Summary*** in English and Dutch concludes this thesis.











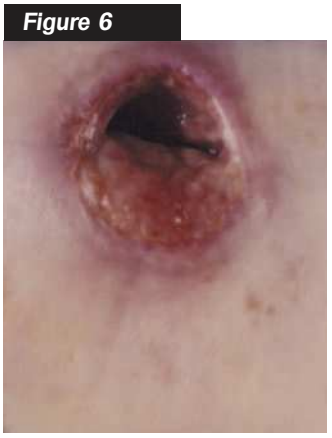


Figure 1. Decubitus ulcer stage I
Figure 2. Decubitus ulcer stage II
Figure 3. Decubitus ulcer stage III
Figure 4. Decubitus ulcer stage IV
Figure 5. Relatively small superficial skin lesion
Figure 6. Mild cutaneous traction, reveals extensive sinus formation (stage IV)

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Chapter 2

Efficacy of Low Level Laser Therapy on Wound Healing in Human Subjects: A Systematic Review

Cees Lucas, RPt[1]; Rob W. Stanborough RPt[2];
Carolyn L. Freeman, RPt[2]; Rob J. de Haan, PhD[3]

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[1] Department of Research and Innovation in Health Sciences, University of Professional Education: Hogeschool van Amsterdam, The Netherlands.
[2] Assigned as Student Assistent, Institute of Physical Therapy, University of Professional Education: Hogeschool van Amsterdam, The Netherlands.
[3] Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, University of Amsterdam, The Netherlands.

Abstract

Objective This systematic review summarises the efficacy of infrared Low Level Laser Therapy (LLLT) on wound healing in human subjects.

Method In order to retrieve randomized clinical trials, we performed computer aided searches of databases (MEDLINE, EMBASE, CINAHL, SPIE, and the Cochrane Database) and of bibliographic indexes. Furthermore, congress reports, reviews and handbooks were checked for relevant citations. Subsequently, all retrieved and blinded studies were scored on methodological quality.

Results We found 4 randomized clinical trials that investigated the effects of LLLT versus placebo or any other intervention. Only one trial demonstrated a beneficial effect. Overall, study quality ranged from poor to insufficient. Of three studies we could perform a meta-analysis. The overall effect size estimate indicates that Low Level Laser Therapy had no significant beneficial effect on wound healing (pooled RR=0.76; 95% CL 0.41 to 1.40).

Conclusions We conclude that there are no scientific arguments for routine application of infrared (904 nm) LLLT on wound healing in patients with decubitus ulcers, venous leg ulcers (ulcus cruris), or other chronic wounds.

Introduction

A decubitus ulcer (in Anglosaxon literature also referred to as 'bedsore' or 'pressure sore') is a problem that has been known since ancient times. The term 'decubitus' is derived from the Latin phrase 'gangraena per decubitum', which means: 'tissue necrosis resulting from lying down'. The current definition of a decubitus ulcer -'any degenerative change, caused under the influence of pressure and shear forces acting upon biological tissues'- also implies that there is a relationship between the development of decubitus ulcers and exposure to pressure, nearly always in connection with illness and immobility. [1] Concentrating on providing good care has taught us that in considerable number of cases decubitus ulcers could have been prevented, which gradually gave rise to the concept that the development of decubitus ulcers could be attributed to inadequate care. [2]

The fact that decubitus ulcers can ***not*** always be prevented, emerged more or less as a new element in the 1992 consensus texts of the CBO (Dutch abbreviation for 'National Organization for Quality Assurance'). [3] This type of statement is obviously not optional, and implies that extra alertness must be maintained in daily practice, and that treatment methods have to be evaluated for their efficacy more than has been done so far.

In recent decades, the problem of decubitus ulcers has gained increasing attention, which is justified in view of the burden imposed on patient and society by decubitus ulcers. [4] Apart from being an obvious burden for the patient, decubitus ulcers are also an economic burden for society. The total costs involved in the treatment of decubitus ulcers in the United States have been estimated at $ 6.4 billion annually in 1994 [5] up to $ 8.5 billion annually in 1997 [6], which is more than the cost of treating patients with AIDS and almost half the amount spent on caring for patients with dementia. [5] The cost of healing an individual decubitus ulcer has been estimated to be between 2,000 and 30,000 US dollars. [7] In the Netherlands the cost of decubitus ulcer prevention and treatment is estimated at $ 700 million per year (1991), the amount spent on treatment vastly exceeding that spent on prevention. [8]

In the United States, the annual number of patients who develop a decubitus ulcer is estimated at 1.7 million. [9] Among patients in skilled care facilities and nursing homes, the prevalence ranged from 2.4 percent to 23 percent. [10-15] Several specific populations may be at higher risk for the development of decubitus ulcers than the general hospital population. Prevalences of 66 percent have been found among elderly patients admitted for femoral fracture. [16] In Dutch nursing homes, where patients are admitted for various reasons (chronic care, rehabilitation [e.g. following an operation or stroke], terminal care) decubitus ulcer prevalences of 15 to 20 percent have been reported [3] (including the 'non-blanching hyperaemia', which is considered to be a stage I decubitus ulcer in the Netherlands).

Facilitating the healing of decubitus ulcers (and other chronic wounds) is an important aspect of the treatment provided by physical therapists, nurses, and other clinicians. Toward that end, a number of treatments are currently in use, and others are being examined for their potential efficacy. In recent years, increasing attention has been focussed on the use of Low Level Laser Therapy (LLLT) as a treatment for open wounds. The first study to document the biologic effects of LLLT was conducted by Mester in the early 1970 's. [17] His work sparked interest in this method particularly in Eastern Europe. More recently, additional work has been published on the subject throughout the rest of Europe and in North America. Many of the conclusions drawn have been supported by studies of mice, [17, 18] rats, [19, 20] and pigs. [21-23] But the question has to be asked, has sufficient proof been reported on human subjects ? In Northern Ireland, one study reported that 64.9 percent of physiotherapists surveyed identified wound healing as most popular indication for LLLT. Patients there were quoted as expecting better results from LLLT, calling it the 'miracle cure' or the 'magic treatment'. [24] Studies such as these however, must be evaluated for their reliance or failure using the accepted standard for clinical studies: the randomized clinical trial (RCT). This design is considered the paradigm for intervention studies because of its potential to provide a valid assessment of the efficacy of an intervention. [25-27]

In this systematic review we summarize the results of randomized clinical trials on the efficacy of infrared low level laser use (≈ 820-950 nm) on wounds in human subjects. [28-31] We also evaluated the quality of available trials according to generally accepted methodological requirements for intervention research [32-37].

Methods

Literature search and study selection

We identified relevant publications by means of computerized searches and citation tracking. The search strategy included MEDLINE (Pubmed), EMBASE, and CINAHL [Cumulative Index to Nursing and Allied Health Literature] (Ebsco) for the period 1975-1998 and was carried out as a double retrieval. Keywords used were: LLLT, low level laser therapy, laser therapy, laser treatment, infrared laser, decubitus ulcer, pressure ulcer, pressure sore, leg ulcer, wound care, and wound healing. In addition, all seemingly relevant MEDLINE 'related articles' were screened for additional meaningful references. All of the retrieved article references were further examined for additional publications. We also checked the Database of the Cochrane 'Rehabilitation & Related Therapies Field'. Furthermore, abstracts, congress reports, reviews, handbooks and unpublished studies were checked for relevant citations. Finally we checked the Database of SPIE, The International Society for Optical Engineering.

The studies had to meet the following criteria to be included in this review: (1) publications had to be written in the English, German, French, or Dutch language; (2) the

studies had to include human subjects with topical ulcerations or wounds; (3) at least one of the interventions under study had to include infrared laser therapy; and (4) the study design had to be an explanatory randomized clinical trial (in which a placebo comparison is used to test for efficacy) or a pragmatic randomized clinical trial (in which the experimental treatment is compared with a standard active treatment).

The assessment of the potentially eligible studies for meeting the entry criteria was done independently by two of the authors (RS and CF). In cases of disagreement consensus was sought with the other authors. All selected publications were blinded for author(s), journal identification, results, and conclusions in an effort to minimize reviewer bias.

Assessment of methodological quality of the trials

All retrieved studies were scored on methodological quality.[35] Two authors (RS and CF) independently assessed the blinded publications, with regard to four categories: (1) study population; (2) description of intervention; (3) measurement of outcomes; (4) analysis and data presentation. These four categories were further divided into a set of 17 methodological criteria (A-Q) [Appendix 1]. The scoring system for these criteria are presented in Appendix 2. Disagreements with respect to methodological quality scores were identified and resolved in a consensus discussion, while the publications remained blinded. If consensus could not be reached, a third (not blinded) reviewer (CL) made the final decision. The final quality score for each study was based on full consensus between the reviewers.

Outcome assessment

With regard to the methodological section a weight was assigned to each criterium relative to its importance for validity, precision or clinical relevance. For each study, a quality score was calculated by summing the weights for all criteria satisfied.

Although all authors used the wound size as an outcome measure, their methods and the amount of published information were very diverse and made an overall effect estimate of laser therapy difficult. In order to obtain all outcome data available, every author received a request (by normal post, E-mail, as well as fax) to provide us with all the data which they possessed in order to perform adequate comparison. Finally, we were able to retrieve these data from all authors involved.

Our meta-analysis focused on comparisons of poor outcome between the experimental and control groups. To maximize the clinical interpretation of intervention outcomes, we defined poor outcome as the number of patients not responding on the treatment (still open wounds at the end of the trial period). For each study we calculated the Relative Risk (RR) for poor outcome. Additionally, we assessed the overall effect size of low level laser therapy by pooling the calculated RRs of the individual studies. In case the X^2-analysis showed the pooled data

to be heterogeneous, we used the random effects model of Der Simonian and Laird, as described by the research group of Ioannidis. [38] If no heterogeneity was demonstrated, we used a fixed effects model (Mantel-Haenzel risk ratio method). [39] In all analyses Relative Risks were expressed with their 95% confidence limits.

Results

Literature search and study selection

The literature search yielded 4,193 publications for skin ulcers (pressure ulcers, decubitus ulcers, and pressure sores), 6,333 for leg ulcers, 1,055 for wound care and 35,269 for wound healing. Combined with laser therapy, LLLT, low level laser therapy, infrared laser therapy, and laser treatment on ***human subjects*** the number of studies were reduced to eight studies and combined with randomized clinical trial reduced to only four RCT's. Two studies involved venous leg ulcers (ulcus cruris) [29, 31], one study [28] described pressure ulcers (decubitus ulcers), and one study [30] reported on various types of skin ulcers and delayed post-operative healing. Of the four articles that satisfied our conditions for inclusion in the blinded analysis, two were identified from the electronic databases [28, 29] and two from reference tracing [30, 31].

Assessment of methodological quality of the trials

The methodological scores ranged from 29 to 47 (maximum score = 100). The median score was 39 points, indicating the overall poor methodological quality of the trials. Only the descriptions of drop-outs (E), informative description of treatment (G), and co-interventions avoided, or comparable (J) were most complete and in general satisfactory. The descriptions of the other criteria were rather poor [Table 1]. The four studies [28-31] explicitly mentioned that the allocation procedure was randomized, but three of them [28, 30, 31] failed to mention how this was done or if the method of randomization was concealed.

Table 1

Results of the methodological quality scores of the included randomized clinical trials

Criteria	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	Methodological
	5	5	15	10	5	5	10	3	3	4	4	4	8	4	5	8	2	score (max.100)
Study:																		
Nussbaum [28]	3	-	-	6	5	-	9	-	3	3	-	4	4	2	3	5	-	47
Malm [29]	3	5	-	4	5	-	8	3	-	3	4	4	4	2	-	-	-	45
Iusim [30]	-	-	-	4	5	4	6	-	3	1	1	1	4	-	3	2	2	33
Bihari [31]	1	-	-	2	2	1	6	3	-	3	1	1	4	-	5	-	-	29

- = no points given for this criterium (poor quality)

Outcome assessment

Table 2 shows a detailed description of the study design, method, participants, interventions and outcome measures. The studies are arranged according to their methodological quality score. Three studies [28-30] reported no effect of laser therapy, whereas one study [31] reported efficacy of laser therapy in wound healing. It is noteworthy that the 'negative studies' have a better methodological score. Two out of the three negative trials [28, 29] however, were hampered by rather large drop-out rates of 20% and 24% respectively. The positive trend of the remaining study [31] was flawed by many co-interventions. The study reporting a favourable outcome of laser therapy (according to the authors) used almost identical dose compared to the studies with a negative result. The use of somewhat different dosages per study could not be related to the outcome. No major complications or side effects were reported in the four trials presented in this review.

With regard to our meta-analysis we only could calculate (pooled) Relative Risk estimates in three trials. [29-31] One negative trial [28] was excluded for this analysis, because its outcome measure was defined as time needed to complete wound healing (survival analysis). Consequently, this outcome measure was incomparable with the others since our definition of poor outcome (the number of patients not responding on the treatment) could not be applied. As can be seen from table 3, two trials showed a Relative Risk reduction of poor outcome of 12% (100%[1-RR]), whereas one trial demonstrated a Relative Risk reduction of 83%. The overall effect size estimate indicates that Low Level Laser Therapy had no significant beneficial effect on wound healing (pooled RR=0.76; 95% CL 0.41 to 1.40).

Discussion

The use of laser devices for healing wounds is becoming increasingly attractive to physical therapists. [28] A number of animal [17-23] and in vitro [40-43] studies have claimed that laser irradiation has a significant effect on components of tissue repair. Conversely, some other (animal) studies showed no significant differences in healing between laser-treated wounds and untreated control wounds. [44, 45] The technical settings and dosage parameters, however, that should be used to produce a positive effect in patients are still uncertain. [41] Many existing studies provide incomplete details of treatment characteristics, making this research difficult to replicate. [22, 46] Anecdotal reports of successful laser treatment of human wounds are plentiful, but controlled human studies scarcely appear in the literature. Much of the previous work does not compare lasers with an alternative (physical therapeutic) modality. [47-50]

Research on LLLT has depended mainly on animal wounds consisting of surgically excised skin. These wound models excluded common problems associated with delayed healing, such as ischaemia, infection, necrotic debris, loss of large amounts of subcutaneous

Table 2

Detailed description of studies and outcomes

Study (Ref)	Method	Participants	Intervention	Outcome measures	Notes
Nussbaum et al, 1994 (28)	Randomized trial, observer blinded, no intention to treat analysis Methodological quality score 49	20 spinal cord injured patients with 22 pressure ulcers Mean age 40 (range 15-61) No duration of illness described	Laser treatment 3x/week with a clusterprobe wavelength 820 nm (1), 660 nm (10), 880 nm (10), 950 nm (10) radiant exposure 4 J/cm² av. power 15 mW repetition rate 5000 Hz pulse duration 160 nsec treatment time 35 sec technique contact treatment or US/UVC treatment, or control treatment	Mean weekly healing rate: Control group (n=6) 32.4% NS US/UVC group (n=6) 53.5% S (p=0.032) Laser group (n=6) 23.7% NS Overall (n=18) 36.54% Measured at initial assessment and every 2 weeks until wound closure	No effect 20% selective drop out rate Data do not allow for pooling
Malm et al, 1991 (29)	Randomized trial, therapist, patient, and observer blinded, no intention to treat analysis Methodological quality score 45	42 patients with venous leg ulcers Laser group (n=21, 11f, 10m) mean age 60 (range 43-77) Placebo group (n=21, 12f, 9m) mean age 61 (range 46-76) No duration of illness described	GaAs Laser treatment 2x/week for 12 weeks max. wavelength 904 nm radiant exposure 1.96 J/cm² av. power 4 mW peak power 10 W divergence 70 mrad repetition rate 3800 Hz pulse duration 180 nsec treatment time 10 min technique contact treatment or placebo treatment	Baseline ulcer area: Laser group x̄ 12 cm² (range 4-52) Placebo group x̄ 14 cm² (range 3-44) Mean and end measurement not given / no data '... enough patients were studied to detect a 40% increase of ulcer healing with 80% power (p<0.05)'	No effect 24% drop out rate
Iusim et al, 1992 (30)	Randomized trial, inadequately blinded, baseline correction/selection, no intention to treat analysis Methodological quality score 33	21 patients (10f, 11m) with 31 skin ulcers and delayed post-operative wound healing Red LLNB group (n=9) Infrared LLNB group (n=11) Placebo group (n=11) Mean age: Red LLNB 71 (range 57-85) Infrared LLNB 74 (range 44-88) Placebo 75 (range 60-87) No duration of illness described	Laser treatment on a daily basis (x̄ 19 days) Red LLNB / Infrared wavelength 660 nm / 904 nm av. power 7.5 mW / 2.7 mW peak power 75 mW / 270 mW max power cont.mode 18 mW / 25 mW duty ratio 10% / 1% focus area 2 cm / 1.5 cm repetition rate 100 Hz / 100 Hz treatment time 7 min continuous mode and 7 min pulsed mode or placebo treatment	Baseline / End / %HR / nr. tr. Red group x̄ 3.2 cm² / x̄ 0.39 cm² / 89 / x̄ 20 Infrared group x̄ 4.72 cm² / x̄ 1.64 cm² / 58 / x̄ 17 Placebo group x̄ 3.8 cm² / x̄ 1.61 cm² / 41 / x̄ 19 t-test: p between Red and Placebo 0.0345 S p between Red and Infrared 0.09 S p between Infrared and Placebo 0.46 NS Measured at initial assessment and every 10 days Photo's were taken at start of treatment, every 15 days during treatment, and at the termination of the treatment	No effect for 904 nm Infrared Possitive effect for 660 nm Red light Pre-post comparison of data Incorrect allocation procedure suspected Many co-interventions (including a well balanced diet)
Bihari et al, 1989 (31)	Randomized trial, patient and observer blinded, no intention to treat analysis Methodological quality score 29	45 patients with crural ulcers, resistant to conventional therapy,divided over 3 groups (n=15) No mean age described No duration of illness described	Laser treatment 1x/week HeNe / HeNe+IR / Noncoherent, nonpolarized filtered light (placebo) wavelength 632.8 / + 904 nm / not given rep..rate cont. / 4800 Hz / cont. technique man.sc. / machine sc. / projection rad.exp. maximum 4 J/cm² for all categories	Measured at initial treatment; last measurement at 9 months, other measurements unclear Ratio's given on wound healing: effective / not effective HeNe 14 / 1 HeNe + IR 14 / 1 Placebo 8 / 5	Positive effect Co-intervention of adjuvant therapy under the same protocol for all groups, including elastic compression bandaging and antibiotics

Key to abbreviations:

av. power	=	average power	min	=	minutes
US	=	ultrasound	f	=	females
UVC	=	ultra violet C	m	=	males
GaAs	=	Gallium Arsenide	n	=	number
HeNe	=	Helium Neon	nm	=	nanometer
IR	=	infrared	mW	=	miliWatt
LLNB	=	Low Level Narrow Band (Light)	W	=	Watt
man. sc.	=	manual scanning	Hz	=	Herz
machine sc.	=	machine scanning	nsec	=	nanosecond
rep. rate	=	repetition rate (pulse frequency)	J/cm^2	=	Joules per square centimeter
rad. exp.	=	radiant exposure (energy density, ED)	NS	=	not significant
cont.	=	continuous	S	=	significant

tissue, sinus formation, and induration of surrounding tissue. [51] Therefore, animal wounds that consist of lineair incisions may be inappropriate models for studying laser effects on chronic wounds.

Different nursing regimens are also known to influence the rate of healing, and optimum clinical conditions appear to be dependent on a moist wound surface. [52] When wounds are allowed to dry out, viable tissue is subjected to secondary desiccation. None of the studies on LLLT involved, mentioned nursing regimens specifically.

Table 3

Results of the meta-analysis (n=3 randomized clinical trials); effect sizes expressed in Relative Risk (RR) estimates with 95% Confidence Limits (CL)

Author	Year	Total Patient Group	Intervention		Control			95%CL	
			Poor	Good	Poor	Good	RR	Low	High
Bihari [31],	1989	28	1	14	5	8	0.17	0.02	1.30
Malm [29],	1991	32	4	13	4	11	0.88	0.27	2.93
Iusim [30],	1992	22	7	4	8	3	0.88	0.49	1.55
							Pooled RR*	95% CL	
Total		82	12	31	17	22	0.76	0.41	1.40

*X^2 test p=0.31; Random Effects Model used.

Our review indisputably demonstrates major methodological shortcomings in randomized clinical trials evaluating the efficacy of low level laser therapy on wound healing in human subjects. The small size of the study population is a frequent problem in laser therapy research in general, and in this review particularly. For this reason, studies may lack the statistical power to detect clinically relevant treatment effects. Another problem with small sample sizes is that important (un-)known prognostic variables might not be in balance between the study groups after randomization. Such situations may lead to biased outcomes if, by chance, the patients in one group had a more favourable prognosis. Indeed, it has been stated that a medical experiment that is not properly designed and carried out must be considered unethical. [53] The problem is not, by any means, confined to the LLLT literature [54], but even the most cursory examination of papers published in this field will reveal reports of experiments carried out without proper controls [47-50], with samples too small to give a statistically significant result [48, 49], and with inappropriate statistical analysis of the data. [31, 48] The results of our meta-analysis on three trials [29-31] do not support the claim of effectiveness of low level laser therapy on wound healing in human subjects. Since the excluded trial for the meta-analysis did not demonstrate a beneficial effect [28], this exclusion will not be of influence on the final results.

Our failure to find a stimulating effect for infrared low level laser therapy is surprising because physical therapists' clinical impression is that it is effective for wound healing. [24] The problem of sorting out optimum treatment characteristics for LLLT may be complicated because of the large number of variables. Clinical results may be dependent on wavelength, pulse duration, irradiance (W/cm^2), radiant exposure (J/cm^2) [or energy density (ED)], power density, pulse repetition rate (frequency), treatment time, treatment repetition rate, or a combination of all of these factors.

At present, we conclude that there are no scientific arguments for routine application of low level (infrared) laser therapy on wound healing in patients with decubitus ulcers, venous leg ulcers (ulcus cruris), or other chronic wounds. Clinical treatment decisions for patients affected with these wounds can only be improved by additional evidence from further clinical research.

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Appendix 1.

Criteria list for assessing the methodological quality of randomized clinical trials of low level laser therapy

Criteria *		Weighting
Study population		**45**
A	Selection and restriction	5
B	Adequate randomization procedure	5
C	Study size	15
D	Comparability of relevant prognosis at baseline	10
E	Drop-outs	5
F	Loss-to-follow up described for each treatment group separately	5
Interventions		**20**
G	Informative description of treatment(s)	10
H	Placebo controlled study	3
I	Pragmatic control group included	3
J	Co-interventions avoided (or comparable)	4
Measurement of outcome		**25**
K	Blinding of patient	4
L	Blinding of physician (therapist)	4
M	Relevant outcome measures	8
N	Blinded outcome measurement	4
O	Adequate follow-up period	5
Analysis and presentation of data		**10**
P	Adequate analysis and presentation of results	8
Q	Adequate adjustments for confounding variables	2
Total		**100**

* Further details are given in Appendix 2.

Appendix 2. Scoring criteria listed in Appendix 1.

A	One point each if criteria for selection is clearly described, restriction to a homogeneous population with respect to diagnosis, duration of complaint, previous treatments, and contra-indications for the treatment of infrared laser.
B	Five points if the randomization procedure is described and is a procedure which excludes bias.
C	Five points if smallest group is larger than 25 patients immediately following randomization; 10 points if larger than 50 patients; 15 points if larger than 75 patients.
D	Two points each if the study groups are comparable at baseline for (1) duration of complaint; (2) age; (3) baseline scores for outcomes measured; (4) recurrence status; (5) previous treatment of complaint.
E	Five points if there are no drop-outs after randomization. Two points if there are drop-outs with the number of drop-outs given for each study group. Three additional points if the reason for withdrawal after randomization is given for each study group.
F	Loss-to-follow up: {1 minus (the number of patients at the main moment of effect measurement / the number of patients at randomization)} x 100%. One point if loss-to-follow up is less than 20% in each group; 4 points if it is less than 10% in each group.
G	Points are given fo a description of the treatment, 1 point each: (1) type of laser used; (2) wavelength and repetition rate [pulse frequency]; (3) duty cycle; (4) power; (5) irradiation [intensity]; (6) distance of probe to skin or contact; (7) monolaser or multilaser; (8) treatment time and - frequency; (9) probe position to skin, angular or perpendicular; (10) misc., plastic foil used for hygienic reasons, alcohol use, etc.
H	Three points if a comparison is made with a study group receiving a placebo treatment only.
I	Three points if a comparison is made between two or more existing interventions.
J	One point if co-interventions are comparable between the groups; 3 points if co-interventions are standardized or avoided in the study design.
K	One point if blinding of patients was attempted, 3 additional points if the blinding proved to be successful.
L	One point if blinding of therapists was attempted, 3 additional points if the blinding proved to be successful.
M	Points for assessed outcome measure: 2 points for pain; 4 points for global measure of improvement (decreased wound surface area), and 2 points for adverse reactions.
N	Points for every blindly assessed outcome measure: 1 point for pain; 2 points for global measure of improvement (decreased wound surface area), and 1 point for adverse reactions.
O	Three points if the timing of effect measurement is identical for all study groups. Two additional points if final effect measurement was made at least 3 months after randomization.
P	Two points for intention-to-treat analysis. One point if data for most important outcomes measure on the most important moment of effect measurement are adequately presented (frequencies, mean, standard deviation). One additional point for an adequate analysis with adjustments for drop-outs, loss-to-follow up, missing values, non-compliance and co-interventions if appropriate.
Q	Two points for having adequate corrections for confounding variables.

Chapter 3

The Effect of Low Level Laser Therapy (LLLT) on Stage III Decubitus Ulcers (Pressure Sores); a Prospective Randomized Single Blind, Multicenter Pilot Study

Cees Lucas, RPt[1]; Cees H.M. Coenen, PhD[1]; Rob J. de Haan, PhD[2]

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[1] Department of Research and Innovation in Health Sciences, University of Professional Education: Hogeschool van Amsterdam, The Netherlands.

[2] Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, University of Amsterdam, The Netherlands.

Abstract

Objectives This article describes a randomized pilot study in four nursing homes. The objectives of this pilot study are: [a] to assess the feasibility of a multicenter trial in a nursing home setting; [b] to investigate whether the type of evaluation method is applicable; [c] to assess the extent of wound size reduction in both treatment arms for an adequate power analysis for future trials; and [d] to analyze the treatment effect(s) of a gallium aluminium (GaAl) 904 nm cluster laser (consisting of 12 infrared diodes) at a radiant exposure (energy density) of 1 J/cm^2 on tissue repair of full thickness stage III pressure sores.

Subjects A total of 20 patients were enrolled into the study, 16 patients were randomized, and four patients were excluded.

Methods Treatment was the prevailing consensus decubitus treatment (n=8); one group (n=8) had 904 nm LLLT in addition, five times a week over a period of six weeks. The main outcome measure was the median wound size at six weeks after intervention.

Results No statistical significant difference was found in wound size ***between*** the two groups (Mann Whitney *U* Test; p=0.47). The median wound size reduction compared to baseline was 83% in the LLLT group and 95% in the control group. There was a significant wound decrease ***within*** treatment arms (Friedman Two-way Analysis p<0.001).

Conclusions It was concluded that a multicenter study is feasible in nursing homes, whereas the evaluation methods turned out to be easy and accurate. A large scale clinical trial is needed to demonstrate the efficacy of LLLT. In preparation of such a trial, we calculated that a sample size of at least 74 patients (37 subjects per treatment arm) would be necessary to detect an average improvement of log 0.3 delta in favour of the experimental group with a two-tailed level of significance (alpha) of 0.05 and a power of 0.80.

Introduction

Of all the perils inherent in prolonged bedrest and immobilization the occurrence of pressure sores remains among the most persistent, especially in an aging population. Measures aimed at prevention have only partially succeeded in reducing the prevalence[1], and its treatment presents a continuing challenge to clinicians. The broad range of products and interventions for treating decubitus ulcers suggests that no standard successful evidence based treatment protocol consists. A trial and error approach to the choice of treatment underscores the poor response to current methods.[2]

The decubitus ulcer is a defect that involves the skin, subcutaneous structures, and/or the adjacent tissue.[3] It may extend to muscle, if present, or to bone. The ulceration may become infected, and the area may be necrotic. The defect has been labeled a decubitus ulcer, pressure ulcer, pressure sore or bed sore.[4] The primary cause is pressure, but numerous other factors can contribute to the formation of decubitus ulcers, including; decreased sensation, poor arterial circulation, muscle atrophy, malnutrition and moisture stemming from perspiration or incontinence.[5] Although pressure has been shown to diminish blood supply to the tissue, the exact amount of pressure applied for a specified time span has not been established.[6] Some studies however, have demonstrated that 70 mmHg applied for 2 hours already may lead to dermal damage and 80 mmHg to frank necrosis.[4]

The prevalence of pressure sores varies considerably in hospital populations but is particularly high among spinal cord injured patients (60%) and elderly immobilized patients (66%), with the incidence increasing with the length of stay.[7] Moreover, a chronic wound, once present, will show delayed healing.[8] Recent figures from the Netherlands show that decubitus ulcers occur in 15-20% of all nursing home patients; in 8-10% of all hospital patients and in 30-50% of all patients with spinal cord lesions in rehabilitation centers.[9] We treat approximately 13,000 patients a year. It is estimated that this amount would double if patients residing at home are included.[9]

Prevention and treatment of decubitus ulcers is extremely expensive and with respect to the rapid increase of the ageing population in Western Europe and the USA is a matter of great importance. In 1992 the cost of decubitus ulcers in the Netherlands was estimated at Hfl 700 million (≈ € 320 million) in the inpatient (hospital health care) and at the same amount in the outpatient population (on an extramural basis).[10]

Although there is opinion based consensus in prevention and treatment,[11] clinicians must continue to search for ways which will induce a faster wound healing. In consensus-texts concerning decubitus ulcer treatment there is a distinction between treatments which are (expected to be) ***effective***, treatments which are ***probably effective*** and treatments which

are ***useless***.[11] It is remarkable that the applications of treatments like ultrasound (US), ultraviolet radiation (UV), and iontophoresis (Xanthinol-nicotinate) have been exclusively mentioned in the 'probably effective'-category (US) or the 'useless'-category (UV and iontophoresis). In physiotherapeutic references however, these applications are still mentioned as effective.[12, 13]

Low Level Laser Therapy (LLLT) has been thought to have an influence on the rate of wound healing.[14] The effects of LLLT on various cell types involved in the healing process have been examined in theory as well as in vitro, but little research has been done in vivo.[15] Based upon these few studies, a number of possible mechanisms of action of laser-mediated photobiomodulation have been postulated and studied. These 'laser catalyzed reactions' include: acceleration of the inflammatory phase of wound healing [16], enhanced prostaglandin concentration [17, 18], enhanced ATP synthesis [19, 20], enhanced collagen synthesis [20, 21], enhanced fibroblast proliferation [20, 21], enhanced phagocytosis of macrophages [22, 23], activation of the immune system [16], and stimulation of resorption and diffusion [24] resulting in cellular proliferation and acceleration of the wound healing process.

In preparation of a planned clinical trial to assess the efficacy of LLLT in chronic wounds, we performed a pilot study. The objectives of this small sample trial are: [a] to assess the feasibility of a multicenter trial in a nursing home setting, [b] to investigate whether our type of evaluation method is applicable, [c] to assess the extent of wound size reduction in both treatment arms for an adequate power analysis, and [d] to analyze the treatment effect(s) of a gallium aluminium (GaAl) 904 nm cluster laser (containing 12 infrared diodes) at a radiant exposure (energy density) of 1 J/cm^2 on tissue repair of full thickness stage III pressure sores.

Method

Subjects

This preliminary study was conducted as a randomized, single blind, multicenter clinical trial. The study was conducted at four different nursing homes, using the same prospective protocol. Consecutive patients with stage III pressure ulcers were eligible. Decubitus ulcer stage III was defined as a full-thickness skin defect extending into the subcutaneous layers and adipose tissue. [11, 25, 26] Wounds were limited to stage III pressure sores, because ulcers of such classification are well measurable and easy to penetrate with a laser beam. There were no age or sex restrictions for participation in the study.

To obtain an adequate, standardized, and reproducible LLLT treatment, the wound surface area had to be covered by the laser probe completely. Therefore, patients with a

wound surface area greater than 30 cm^2 were excluded. All wound coverings (e.g. hydrocolloid dressings, film dressings, or foams) were removed. Wounds completely occluded by eschar were not included in the study. Patients were also excluded if they had a constant, invariable ulceration for over one year, or if they were at terminal state. In case of diabetes, patients were not included if they suffered from serious metabolic disorders.

After assessment of baseline characteristics (age, sex, decubitus ulcer location, wound duration, Norton score [27], and initial wound size) all patients were randomly assigned to one of the two treatment protocols; the experimental group (Low Level Laser Therapy + consensus treatment), or control group (consensus treatment only). Additional medication which could affect wound healing (e.g. corticosteroids) were not administered and no concurrent physiotherapeutic interventions were initiated during the study.

The patients were participants in the study for a maximum of six weeks or shorter if complete wound healing occurred. The six weeks evaluation period was choosen, because the literature suggests that a meaningful effect on healing would occur in that amount of time. [28] Each included patient (or their representative in case of legal incompetence in psychogeriatric patients) signed an informed consent form. The procedures followed were in accordance with the ethical standards of the responsible committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

Treatment Regimens

All patients received the prevailing consensus decubitus ulcer treatment; whereas one group had LLLT in addition. Consensus decubitus ulcer treatment involved information and instruction of the patient, wound cleansing, simple moist dressings, and frequent alteration of the patients position. Treatments were given over a period of 6 weeks (max.), 5 times a week (except for the weekends).

LLLT treatments were administered using an LLLT device with a microprocessor-controlled, multiple monochromatic optical source probe (Combilaser C-501™ , Schreuder Medical, Amersfoort, The Netherlands). The handheld probe with 12 x 70 Watt monochromatic infrared GaAs-diodes (Gallium Arsenide) operated at a wavelength of 904 nm in a 830 Hz pulse frequency mode with an average beam power of 8 mW and a radiant exposure of 1 J/cm^2 covered an area of 30 cm^2. The instrument is a class III-b laser device manufactured according to safety standard IEC 601.1. Continuous beam power was guaranteed by using laser diodes from one production process which were calibrated for all four devices. Furthermore we did not use the so called 'energy pack', a small 12 Volt dry lead accumulator, but on line current as an energy source. This has the advantage that there is no loss of electric potential if the battery is not completely charged and guarantees equal output. To obtain an energy density of 1 J/cm^2 an exposure time of 2 minutes and 5 seconds (125 sec.) was needed. By means

of an infrared detection device the infrared diodes were checked on their output every two weeks by an investigator not involved in the treatment. Before and after each treatment the cluster probe was cleaned with alcohol (spiritus ketonatus 95%) to prevent cross-infection. All LLLT-treatments were given by the same investigator assigned at each of the four facilities.

Evaluation

Once a week the wound appearance (e.g. colour, presence or absence of necrotic tissue, eschar, and inflammation) and the Norton score were documented. The Norton score is an ordinal scoring system which reflects the at risk situation of patients with respect to pressure ulcers. [27] A score of ≤ 14 implicates increased risk due to decreased activity, mobility, mental and physical status and regular incontinence. Wound surface area was registrated in mm^2 based on a 1:1 Polaroid Image Exposure™ (deviation ≤ 1%). This instant colour photograph was taken by the clinicians every week to provide a permanent time series record. This measurement technique is simple, reproducible [29], and easy to accomplish at the bedside. In addition, an independent and trained evaluator outlined the area of these measurements on a transparant wound diagram consisting of a mm^2 scaled grid. The perimeter of the vital borderline of the ulceration was transposed to the transparency and the enclosed area (mm^2) was determined by the investigator (C.L.), blinded for the clinical details.

Assessments of Clinical Outcome

The primary outcome was the median wound area (mm^2) at six weeks after the intervention started. During the study period, response to treatment was also calculated as a decrease in the surface area of the patient's ulcer. Healing (0 mm^2) was scored as a complete closure of the wound without any residual exudate or inflammation in the dermis.

Statistical Analysis

Baseline characteristics and outcome data were analyzed with non-parametric descriptive statistics. Six weeks after intervention the differences in wound sizes (mm^2) ***between*** the two treatments were compared, using the Mann Whitney *U* Test. We also calculated the median wound size reduction in terms of percentage compared to baseline. With respect to differences ***within*** both treatment groups we performed the Friedman Two-way Analysis.

Results

Twenty patients were enrolled into the study. Eight patients were randomized to consensus treatment, and eight patients had LLLT in addition. We excluded 4 patients before randomization; two because of interference due to medication, one because the wound size did not meet the entry criteria, and one because after removal of necrotic tissue the pressure sore was classified stage IV.

The baseline characteristics were quite similar between the two groups (Table 1). Compared to the control group, the experimental group had a slightly larger initial median wound size and slightly longer decubitus ulcer duration.

Table 1

Baseline characteristics of the study group (n=16)

		LLLT Group (*n*=8)	Control Group (*n*=8)
Age (years)	Median	87.5	88
	Range	73 - 92	72 - 95
Sex	Male	2	-
	Female	6	8
Location	Gluteal	1	3
	Sacrum/Coccyx	2	2
	Calcaneus	2	2
	Med. Fem. Cond.	1	1
	Lat. Malleolus	2	-
Ulcer duration (wks)	Median	4	3
	Range	1-9	1-10
Norton score	Median	12.5	9
	Range	8 - 17	7 - 17
Initial wound size (mm^2)	Median	94	82.5
	Range	9 - 513	30 - 527

After six weeks there was no statistical significant difference in median wound surface area ***between*** the two groups (Mann Whitney *U* Test; p=0.47), although there seemed to be a treatment effect in favour of the control group. After 6 weeks of treatment we found that wounds in the LLLT group healed to a median of 83% of their initial area. During the same period, wounds in the control group healed to a median of 95% of their initial area (Table 2). ***Within*** each treatment arm, the Friedman Two-way Analysis showed significant decrease in wound size area (p<0.001). The actual median wound size area for each week appears to be approximately inversely proportionate to treatment time in both groups, whereas the intervals between the measurement moments were equal. The LLLT group shows consistent decrease of wound surface area, while in the control group there seems to be a deterioration in wound area between the measurement moments of week 2 (t2) and and week 3 (t3) (Table 2 and Figure 1). Figures 2 and 3 present the ***change*** of wound surface areas for each measurement moment of the individual patients for the LLLT group and the control group,

Table 2

Median (mm^2) wound size at each time of assessment and median wound size reduction at six weeks in terms of percentage compared to baseline

	LLLT Group (*n*=8)	Control Group (*n*=8)
Measurement	Median (mm^2)	Median (mm^2)
baseline	94	82.5
week 1	75	40
week 2	60	17
week 3	34.5	34
week 4	28.5	13
week 5	25.5	12
week 6	16	4
Reduction	83%	95%

respectively. As can be seen from Figure 3, two patients in the control group (patient numbers 4 and 6) showed a temporarely increase of wound surface area, affecting the median statistic (Figure 1). No treatment-related adverse effects were reported during this study.

Discussion

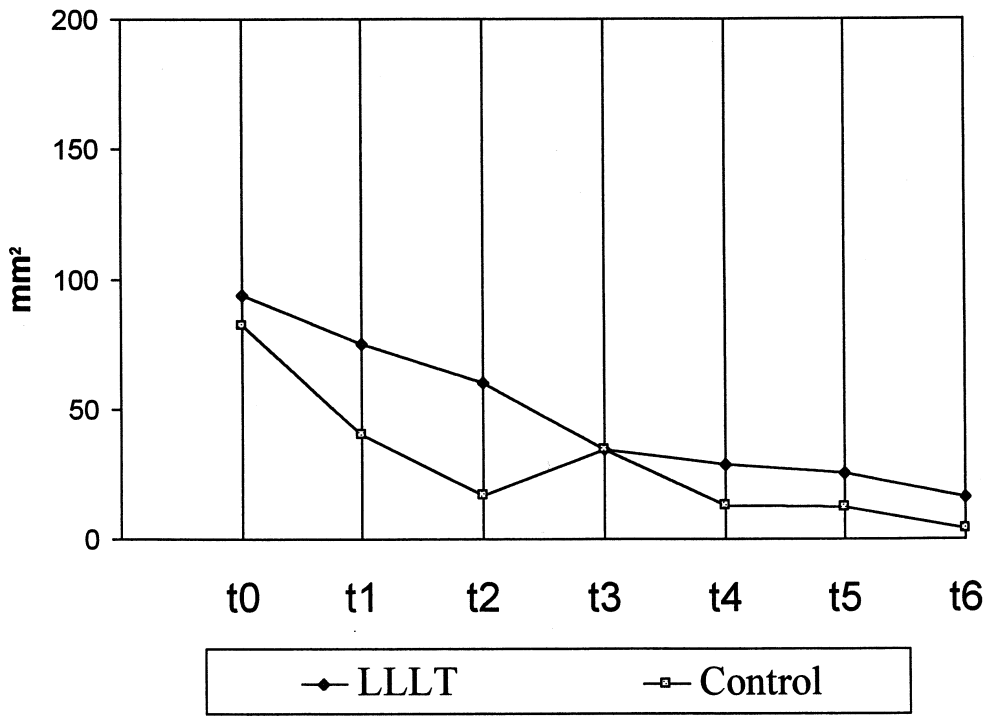
The objectives of this preliminary study were: [a] to investigate whether a multicenter trial was feasible in a nursing home setting; [b] to investigate whether our type of evaluation method was appropriate; [c] to assess the extent of wound area reduction in both treatment arms for an adequate power analysis for a future trial; and [d] to analyze the treatment effect of LLLT.

Our study showed that a multicenter study is very well feasible in a nursing home setting; we did not encounter any specific problems. Enrollment, randomization and blinding procedures were uncomplicated. With respect to the evaluation method, the Polaroid Image 1:1 Exposure technique and the transparent wound diagram technique appeared to be easy and accurate.

The results of theoretical and in vitro studies showed that LLLT enhances the rate and extent of healing of chronic wounds. [19, 30-34] Other studies demonstrated that low energy laser irradiation can be used to promote healing of acute wounds induced in animals [31, 35 -38] and the healing of venous leg ulcers in humans. [39, 40] Conversely, some other animal studies showed no significant differences in healing between laser-treated wounds and untreated

Figure 1

Median wound surface area for each measurement moment
Comparison of the LLLT group (n=8) and the control group (n=8)

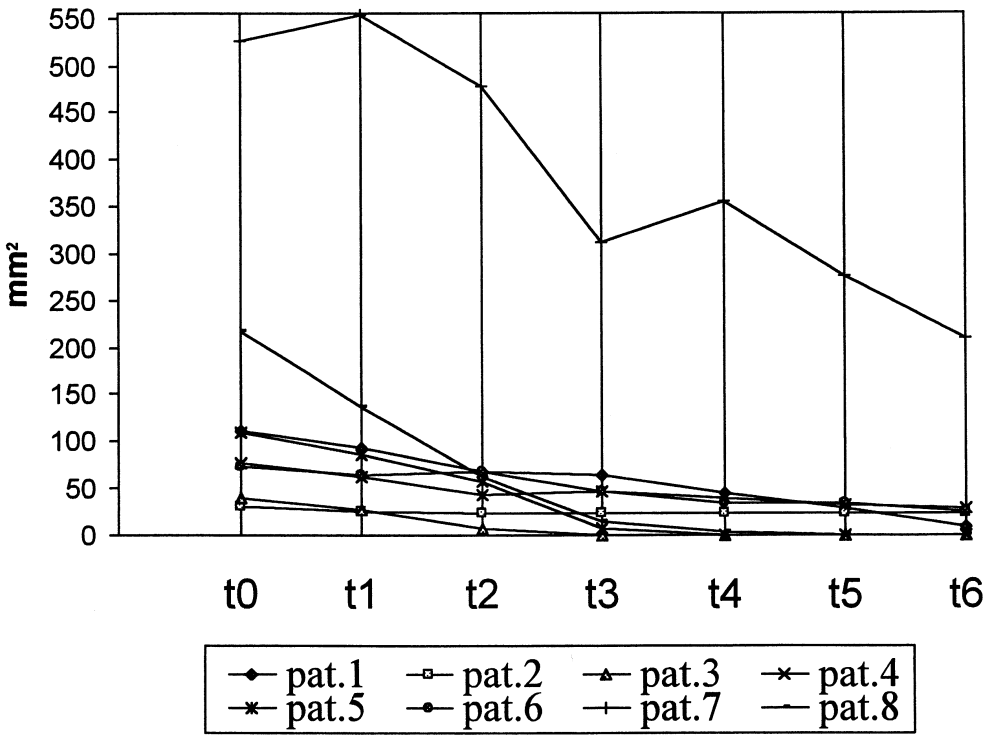


control wounds.[41, 42] Our study results indicate a significant reduction of sore surface area ***within*** both treatment arms. That wounds in the control group healed as well during this study is not surprising. All wounds received an intensive amount of additional care, including the maintenance of a moist wound microenvironment and all other consensus interventions as part of the treatment protocol. The observed temporarely increase of wound surface area in the control group was explaned by the data of only two patients. Therefore, to our opinion, no clinical relevance should be attributed to this phenomenon.

It is noteworthy, that no adverse effects attributable to low level laser therapy were reported during this study. Not surprisingly, in our small sample pilot trial, we could not demonstrate that LLLT in addition to standard care had a favourable effect (if existing) on the wound area compared to standard care only. A large scale clinical trial is planned to distinguish a possible effect of promoting wound healing using laser irradiation on stage III

Figure 2

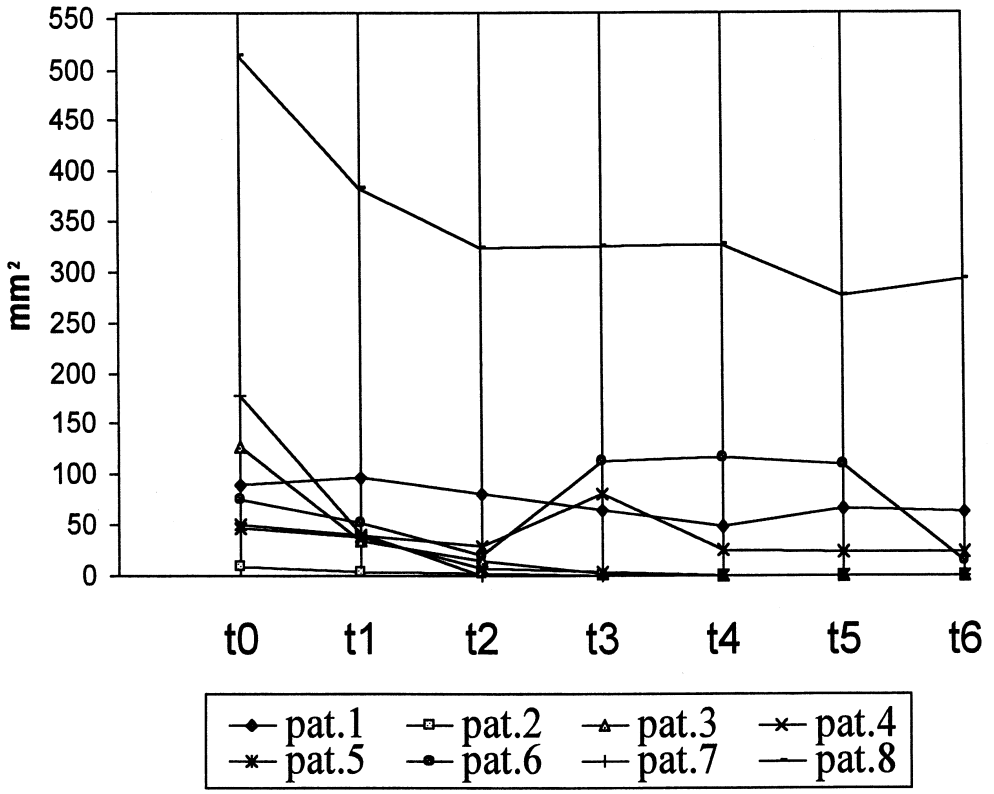
Wound surface area for each measurement moment per patient in the LLLT group (n=8)



decubitus ulcers. Based on the results of this pilot study we calculated that for such trial a sample size of at least 74 patients (37 subjects per group) would be necessary to detect an average improvement of log 0.3 delta in favour of the experimental group with a two tailed level of significance (alpha) of 0.05 and a power of 0.80.

Figure 3

Wound surface area for each measurement moment per patient in the control group (n=8)



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Chapter 4

The Reliability of Wound Surface Area Measurement in Stage III Pressure Ulcers, using an Instant Full Scale Photographic Technique combined with a Transparent Grid Sheet

Cees Lucas, RPt[1]; Jody Classen, RPt[2];
Deannine Harrison, RPt[2]; Rob J. de Haan, PhD[3]

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[1] Department of Research and Innovation in Health Sciences, University of Professional Education: Hogeschool van Amsterdam, The Netherlands.
[2] Assigned as Student Assistent, Institute of Physical Therapy, University of Professional Education: Hogeschool van Amsterdam, The Netherlands.
[3] Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, University of Amsterdam, The Netherlands.

Abstract

Background and purpose An essential element in the treatment of pressure ulcers is the periodic assessment of wound healing. Measuring progress toward healing is fundamental to pressure ulcer management. The purpose of this study was to investigate the intra- and interobserver reliability of an instant full scale photographic technique combined with transparency tracing, avoiding the disadvantages of the separate components of this combination in measuring wound surface area.

Subjects Duplicate photographic measurements of 30 wounds were obtained in 26 patients once a week over a period of two weeks, resulting in 120 photographs in total. Subsequently, duplicate tracing was assessed by two independent observers amounting to 480 observations. Patients were recruited from three long term care facilities.

Methods This study used the Intraclass Correlation Coefficient (ICC) as an indicator of chance-corrected agreement to estimate the reliability for the intra- and interobserver data. Additionally an Bland-Altman plot was constructed to measure the relationship between interobserver differences and wound surface area.

Results Analysis of the data revealed that all measurement comparisons were highly reliable; ICCs=0.99. No statistical differences between measured surface areas could be demonstrated. Linear regression showed a very small, albeit clinically unimportant, association (ß=0.0027; 95% CL 0 to 0.005) between interobserver disagreement and the size of the wound.

Conclusions The described method represents a simple, practical, and inexpensive technique to accurately monitor and evaluate healing of pressure ulcers over time and should be used in preference to separate transparency tracing or photographic techniques. Our results indicate that measurements obtained with this combined method are highly reliable ***within*** and ***between*** observers.

Introduction

Chronic wounds such as pressure ulcers constitute a problem in rehabilitation. To determine the amount of healing in response to treatment, sequential assessments of changes in ulcer size are essential. [1-6] Although a variety of measurements for pressure ulcer healing have been proposed, a gold standard for quantifying day-to-day changes in pressure ulcer healing has not been established for either clinical or research purposes. [1, 7, 8]

Various ***qualitative,*** multidimensional index scores for wound measurement have been described e.g. the Shea Scale [9], the Sussman Wound Healing Tool [9], the Johnson Scale [10], the Sessing Scale [9, 11,] the Wound Healing Scale (WHS) [9, 12,] the Pressure Sore Status Tool (PSST) [13,] and the Pressure Ulcer Scale for Healing (PUSH). [8, 9]
Besides that, there are ***quantitative*** methods of wound surface area measurement, these methods include circumference, or perimeter measurement [14], maximal perpendicular diameter measurement [15], direct tracing [16-21], photography [19-21], computer aided planimetry [17, 18, 22], computer image analysis [23-25], and stereophotogrammetry. [26-29] Wound area combined with wound volume can be measured by quantitative three-dimensional methods, such as ultrasound imaging [30], and a three-dimensional laser imaging system. [31] With respect to volume determination, the use of dental impression materials [14, 25, 32] and linear wound depth measurements [21, 33-35] are described as well.

In experimental research, the use of (two-dimensional) stereophotogrammetry and quantitative three-dimensional measurement methods (which includes both wound area and wound depth, or wound volume) yields high reliability and validity. [26-31] Both techniques, however, are too expensive and cumbersome for clinical practice [20, 29], and frequently take an inordinate amount of time. There is no evidence that states volume to be a more sensitive indicator compared with linear or surface measurements. [14] In cases in which wound measurement is considered, wound parameters recommended for clinical applications are changes in size and surface area. [14, 20, 29] The data currently available in the research literature suggest that measures of ulcer dimensions by means of transparency tracing and photography provide the most valid indicators for monitoring ulcer changes over time and to assess efficacy of pressure ulcer treatment. [36-40] A review of studies with these techniques, however, reveals that each of these methods also has inherent limitations. [39]

Transparency Tracings

Transparency tracing of wound dimensions consists of outlining the wound perimeter on acetate transparent film. Typically, a sheet of acetate is placed over the ulcer surface and the perimeter is traced using an indelible marking pen. Measurement of the area is determined by laying the transparency over grid paper and counting the centimeter squares contained within the traced area. The time required to complete the ulcer tracing and to

count and calculate the the ulcer area is minimal. Sometimes, however, it is difficult to draw directly on the transparent material due to the wound exudate and the clouding of the transparent material itself. Another disadvantage of the acetate tracing procedure is apparent when removing the material; wound damage and contamination are likely to occur. Furthermore sterilization of the transparency is recommended to prevent cross contamination.[22] Unlike the disposable Kundin gauge™, acetates do not permit immediate calculation, but after cleaning can be appended to the patient's chart and provide a morphological record for subsequent comparison. To summarize, direct tracing is simple, inexpensive, consistent, and reproducable, but unacceptable with respect to the wound care itself.

Photography

Photographic measurement of ulcer size uses a camera equipped with a macro lens. A planimeter or digitizing tablet is used to calculate the ulcer surface area. A major advantage of photographic measurement is the provision of a permanent visual record of the ulcer. The photograph identifies not only the physical dimensions of the ulcer but also the type of tissue present on the ulcer surface. Unfortunately, there is a number of technical limitations imposed by photography that reduces its usefulness. Measurement precision can be compromized when the distance between the camera and the ulcer surface is inconsistent. Failure to place the camera at exactly the same distance for each photograph can create the impression that the size of the ulcer has changed when in reality it has not.[39] Similarly, the camera angle in relation to the ulcer may affect the precision of photographic measurements.[41] Furthermore the need for developing and processing a film before measurements on conventional photographs can be made and the uncertainty with respect to the success of the result (e.g. over- or underexposure) contributes to problems in obtaining the needed data in comparison to instant photography.

Planimetry tracings on transparent material (acetate) were highly correlated with measurements obtained from photographic planimetry of the same patient group ($r = 0.98$ - $r = 0.99$).[20, 36] A comparison of ruler (circumference, or perimeter) measurements with transparency tracings showed an intraclass correlation coefficient of 0.97.[37] Another study described the comparison between measurements with a ruler, transparency tracings, and photography.[38] Transparency tracing yielded the highest degree of precision, regardless the size of the ulcer surface area. Ruler measurement was the least precise of the three techniques. The most likely source of error in tracing wounds may be in the tracing itself rather than in determination of the area traced.[16, 39] Acetate tracing and photography are recommended to obtain the most accurate measure of actual wound surface area[40], and in detecting early changes in wound size.[20]

Objective

The objective of this study was to assess the stability of wound surface area measurement using full scale instant photography combined with a transparent grid sheet (mm^2). Using this combined method we intended to reduce the above mentioned limitations inherent to both techniques substantially. Separately, the two methods demonstrated to be accurate, reliable and valid.[14, 20, 36-40, 42] Yet, no data are available on the stability of the combined scores in terms of intraobserver and interobserver reliability. Intraobserver reliability is based on the measurement of the same person on two occasions using the same instrument. Interobserver reliability refers to the score agreement between independent observers measuring a clinical phenomenon with the same instrument at the same time. For this reason we compared the score agreement between the scores as assessed by dual tracing and calculation of wound surface areas of two instant full scale photographs of the same wounds by two independent observers.

Patients and methods

Patients

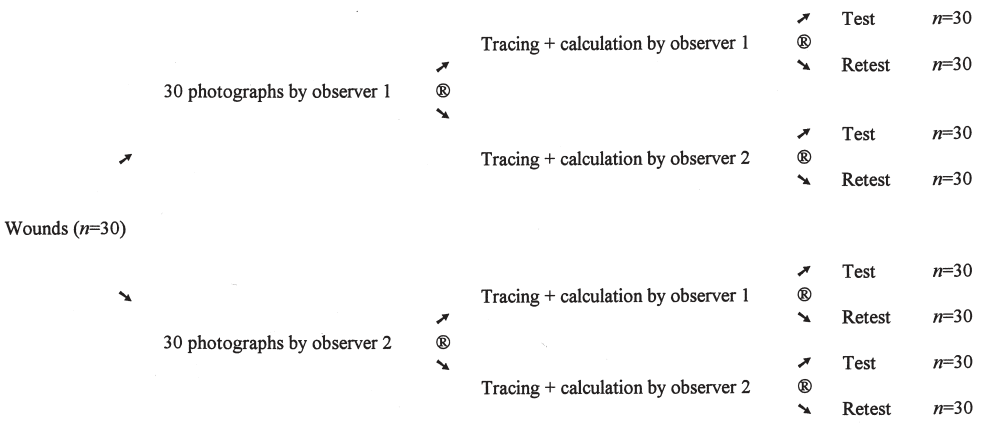
Twentysix patients (with 30 wounds) residing in three long term care facilities in Amsterdam, the Netherlands, participated in this study. Consecutive patients with stage III pressure ulcers were eligable. Decubitus ulcer stage III was defined as a full-thickness skin defect extending into the subcutaneous layers and adipose tissue.[43-45] There were no age restrictions for participation in the study. Critically-ill patients and patients who had clinical evidence of infection in the ulcer were excluded. Wounds that were occluded completely by eschar were also excluded from the study, as well as patients with venous ulcers.
Each included patient (or their representative in case of legal incompetence in psychogeriatric patients) signed an informed consent form. The study was approved by the medical ethical committees of the participating nursing homes.

Methods

Patients were participants in the study for a maximum of two weeks. After baseline assessment (age, sex, location of the pressure ulcers and ulcer duration) their 30 wounds were photographically assessed twice by two independent and trained observers each time, both at baseline and after one week; resulting in a total of 60 x 2 = 120 photographs.
At baseline the 60 photographs, for their part, were assessed twice in random order by the same two observers using a transparent grid sheet, resulting in a total of 240 observations (Figure 1). The same procedure was repeated at week 2, amounting to a total of 480 observations. The intraobserver reliability was assessed by comparing all test-retest results (n=240 paired observations). The interobserver reliability was assessed by comparing all test results of observer 1 with those of observer 2 (n=240 paired observations).

Figure 1

*Photography and tracing scheme for measurements at week one (baseline)**



** The same procedure was repeated in week 2*

Week 1: Camera positioning according to description of anatomical landmarks

Week 2: Camera positioning according to skin marking procedure

® = in random order

The wound surface area was registered in mm^2 based on a Polaroid Image Exposure™. The camera was equipped with a close-up stand to obtain a 1:1 picture (deviation ≤ 1%). This full scale instant colour photography is a simple technique, which does not affect the wound, and is easy to accomplish at the bedside. In addition the two observers outlined the area of the wound surface on a transparent wound diagram consisting of a mm^2 scaled grid. The perimeter of the vital borderline of the ulceration was transposed to the transparency and subsequently the enclosed area (mm^2) was calculated by the two observers independently. The clinical outcome was the wound surface area expressed in mm^2. Complete closure of the wound, which could possibly occur in the second week, was scored as 0 mm^2. In the first week, the camera position was properly described according to anatomical landmarks. In the second week we used an indelible skin marking pen to outline the edges of the rectangular close-up stand on the patients skin to ensure equal camera positioning. After data collection we used a double data entry procedure.

Statistical Analysis

Characteristics and outcome data were summarized with descriptive statistics. For continuous data such as those provided by most wound measurement studies, the traditional measure of intra- and interobserver reliability is the Pearson's Product-Moment Correlation

Coeficient (PMCC). This has also been advocated in standard (para)medical treatises [46, 47] and has been most commonly used in reporting the stability of several measurement techniques. [20] Its disadvantage is that replicate measurements may be systematically different, and yet highly (or perfectly) correlated. [48] In that sense the PMCC may be misleading. A more appropriate approach to assess concordance is the intraclass correlation coeffient (ICC). [49-53] This statistic assesses not only the strenght of correlation, but also whether the slope and intercept vary from those expected with replicate measures. [54] If one measure is systematically higher or lower than the other, the ICC is correspondingly reduced, while the PMCC is not. [50] The ICC can vary from 0.00 to 1.00 where values of ≥ 0.90 are regarded as evidence of high, or excellent reliability; 0.80 - 0.89 as good reliability; 0.70 - 0.79 as fair; and with those below 0.70 indicating poor reliability. [49] All ICCs were calculated using a two-way random effects model. Observation differences between mean values of surface areas were also expressed with their 95% confidence limits (CL).

Additionally, we constructed a Bland-Altman plot [48] of the differences between the two observers against their mean wound surface area assessment to check whether the error of measurements was independent of the size of the wounds. That is, the difference between each pair of observations was plotted against their mean. When depicted graphically, using the *y* axis to show difference scores and the *x* axis to show mean scores, perfect correspondence would be represented by a horizontal line through an ordinate of zero. Any observed differences between the two observers as a function of the range of their mean scores (comparable scores in small wound surface areas, but diverging scores in larger wound surface areas, or vice versa) were taken as evidence of scatter bias. [55-57] The observers disagreement as function of wound size was expressed in a linear regression line. The regression coefficient was also expressed with its 95% confidence limits (CL). All analyses were done with SPSS/PC+ Statistics 8.0 (SPSS Inc, Illinois, USA).

Results

The characteristics of the 26 patients are presented in Table 1. The study population showed a mean wound duration of 4.1 weeks (SD±3.5), and an overall mean wound size of 268 mm^2 (SD±413, Median 111, Range 1-1942). Due to the nursing home setting there is an inevitable overpresentation of elderly women (85%).

In Table 2, both the intra- and interobserver agreement are presented in 95% CL of the differences between the mean values of surface areas, and in ICCs. No statistical differences between the measured surface areas could be demonstrated, whereas all ICCs were high (0.99). The ICCs were not affected by the different camera positioning methods in week 1 and week 2.

The regression coefficient based on 240 paired observations (week 1 and 2) showed a statistical significant, albeit clinically unimportant, association (ß=0.0027; 95% CL 0 to 0.005) between interobserver disagreement and larger sizes of the wounds (Figure 2).

Table 1

Characteristics of the study group (26 patients with 30 wounds)

Age [years (±sd)]	Mean	85.2 (±7.1)
	Median	88
	Range	72-95
Sex	Male	4
	Female	22
Location	Gluteal	7
	Sacrum / Coccyx	7
	Greater Trochanter	1
	Med. Fem. Epicondyle	2
	Lat. Malleolus	3
	Calcaneus	10
Ulcer duration [weeks (±sd)]	Mean	4.1 (±3.5)
	Median	2
	Range	1-13
Woundcategories *		
< 100 mm^2 (±sd) *n*=33	Mean	45 (±30)
	Median	39
	Range	97 (1-98)
100-500 mm^2 (±sd) *n*=16	Mean	202 (±115)
	Median	153
	Range	347 (110-457)
> 500 mm^2 (±sd) *n*=11	Mean	878 (±456)
	Median	670
	Range	1429 (513-1942)

* Overall wound measurement assessed by observer 1 in the first and second week (*n* =2x30 wound assessments)

Discussion

Chronic wounds are those in which simple medical or surgical treatment does not produce easy resolution. [58] Typically, chronic problem wounds are open skin ulcers which lack both the dermal and epidermal layers. They are often irregular in shape and depth due to many episodes of contraction and epithelialization. Frequently these wounds occur over contoured areas of the body and over bony prominences. [40, 58] These characteristics of chronic wounds present difficulty for objective data documentation. However, the accurate measurement of

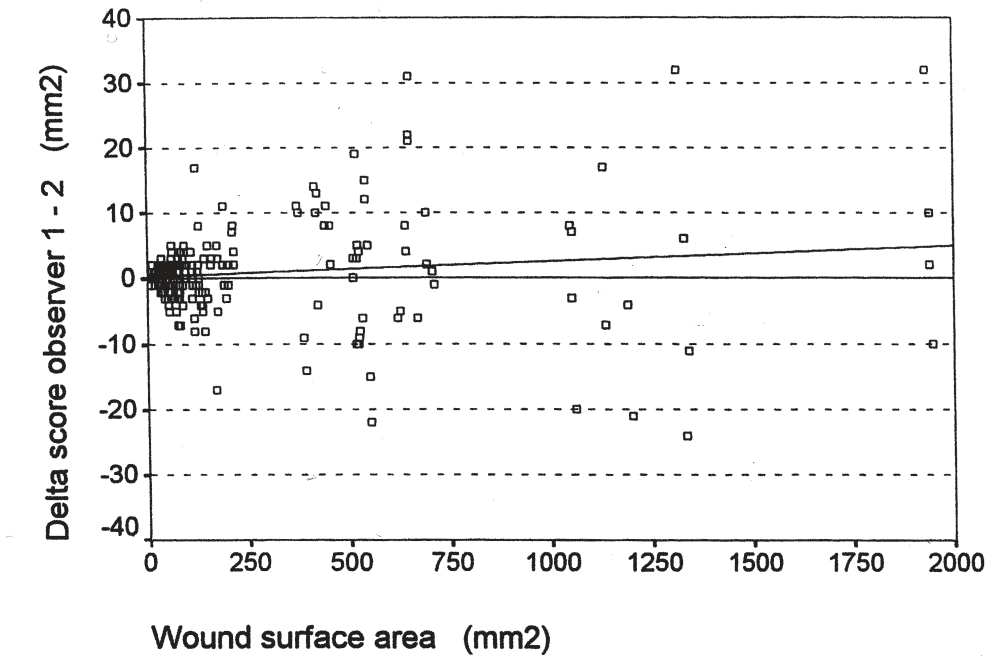
Table 2

Intra- and Interobserver agreement expressed in 95% Confidence Limits (CL) of the differences between the mean values of surface areas (mm²) and in intraclass correlation coefficients (ICCs)

	95% CL of differences between the means	ICC
Intraobserver agreement Observer 1	-1.19 / 0.28	0.99
Intraobserver agreement Observer 2	-0.95 / 0.75	0.99
Interobserver agreement Obs. 1 - Obs. 2	- 0.14 / 1.91	0.99

Figure 2

Bland-Altman plot (n=240 paired observations)
Interobserver difference as a function of wound size area expressed in a linear regression line (ß=0.0027; 95% CL 0 to 0.005)



healing of these wounds is fundamental to evaluate treatment effects in research and to evaluate the rate and quality of healing in clinical conditions [59-61] Further complicating accurate wound measurement is the contamination of the measuring device as it comes in contact with the wound. [40] In our method direct contact of the close-up stand and the

wound surface area could be avoided in all cases, therefore, wound damage and contamination did not occur. In this study all wounds were completely included in one image.

Photographs or tracings of wounds subjected to hand held or computerized planimetry, weighing, and counting blocks on graph paper has been described in various wound studies.[60, 61] These photographs and tracings are two-dimensional and uniplanar which might produce distorsion of three-dimensional multiplanar wound surfaces. From comparison studies it is known that measurements of areas obtained from photographs and tracings slightly over-estimated the area of ulcers when compared to the areas obtained by computer assisted planimetry.[20] However, the cost, the amount of equipment and time required to use this method restrict its clinical usefulness in daily practice.[40]

The current study describes a simple technique, which is easy to accomplish at the bedside in a minimal amount of time. The time required for photography and tracing of one pressure ulcer was < 7 minutes, whereas the cost of materials required for one ulcer measurement is approximately $ 2. Perpendicularity of photography was more or less guaranteed by the width and shape of the close-up stand. Otherwise, the angle must be rather wide to have a substantial impact on surface estimation.[62] In our study this does not appear to be a serious source of imprecision. The camera equipped with the close-up stand guaranteed exactly the same distance for each photograph. By using an instant photography method there was no need for developing and processing a film, and measurements and data were obtained directly. In case of under- or overexposure, the photograph could immediately be taken over, thus avoiding missing data. A full scale instant film system combined with acetate tracing is a noninvasive technique which provides image documentation for both calculation and for visual inspection (colour) over time. The acetate tracings are inexpensive, convenient to use, and provide a permanent graphic representation of the wound. Boundary recognition requires limited training and the tracings can be affixed to the patient's chart. Another advantage of this method is, that it can be used to monitor the wound, even without calculation, because a direct comparison between subsequent tracings can be made by simply laying one tracing over another.

If measures are to be used clinically, besides intraobserver reliability, they should show interobserver reliability as well, because the likelihood of the same person repeatedly rating the same patient is minimal in many clinical settings. The results of this study clearly show that this combined wound measurement method shows excellent intra- and interobserver reliability in subjects with stage III pressure ulcers. The different camera positioning methods in week 1 and week 2 did not affect the outcome. Only a very small variance (2%) in observers disagreement could be explained by the wound size; this relationship may be considered as clinically unimportant. An issue to be examined in further research might include venous leg

ulcers, since the appearance of those wounds is characterized by a more irregular shape in comparison with pressure ulcers.

Conclusion

To be clinically useful, an ideal measurement instrument to assess patients' wound size areas needs to be inexpensive and practical enough to be used regularly in a range of settings by a variety of health care professionals. Such an instrument must be easy to learn, simple to apply, and safe for patients. Moreover, for clinical and research purposes, the instrument should be reliable and valid for measuring wounds of diverse size, location, and appearance. With these needs in mind, the described method of an instant full scale photographic technique, combined with a transparent grid sheet represents an excellent starting point to validate healing of pressure ulcers over time and should be used in preference to single transparency tracing or photographic techniques. The results of this study demonstrate that this method is highly reliable for assessing wound size areas of stage III pressure ulcers.

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Chapter 5

Efficacy of Low Level Laser Therapy in the Management of Stage III Decubitus Ulcers: a Prospective, Observer Blinded, Multicenter, Randomized Clinical Trial

Cees Lucas, RPt[1]; Martin J.C. van Gemert, PhD[2];
Rob J. de Haan, PhD[3]

Submitted for Publication

[1] Department of Research and Innovation in Health Sciences, University of Professional Education: Hogeschool van Amsterdam, The Netherlands.
[2] Laser Centre, Academic Medical Center, University of Amsterdam, The Netherlands.
[3] Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, University of Amsterdam, The Netherlands.

Abstract

Background Low Level Laser Therapy (LLLT) has been suggested as a promising treatment option for open wounds. In view of the absence of randomized studies with sufficient large sample sizes, we assessed the efficacy of LLLT in the treatment of stage III decubitus ulcers.

Methods We performed a prospective, observer blinded, multicenter, randomized clinical trial to assess the effect of LLLT as adjuvent to standard decubitus care. A total of 86 patients were enrolled into the study. Treatment was the prevailing consensus decubitus treatment (n=47); one group (n=39) had LLLT in addition, five times a week over a period of six weeks. The primary outcome measure was the absolute (mm^2) and relative (%) wound size reduction at six weeks compared to baseline. Secondary outcome measures were the number of patients developing a stage IV ulcer during the study period, and the median change in Norton scores at six weeks compared to baseline.

Results Mann Whitney U tests showed that the differences between the two groups in terms of absolute improvement (p=0.50) and relative improvement (p=0.40) were not significant. Because the wound size areas were non-normally distributed, we additionally analyzed the data after logarithmic transformation of the wound size measurements. No significant difference in $\log_e$ improvement scores between both groups could be demonstrated (unpaired t-test: p=0.64). During the treatment period 11% of the patients in the control group, and 8% of the patients in the LLLT group developed a stage IV decubitus ulcer (Fisher's exact test: p=0.72). The patients' Norton scores did not change during the treatment period.

Conclusions In this trial we found no evidence that justifies using Low Level Laser Therapy as an adjuvant to the consensus decubitus ulcer treatment.

Introduction

A decubitus ulcer (pressure ulcer, or pressure sore) is defined as 'any degenerative change, caused under the influence of pressure and shear forces acting upon biological tissues'.[1] In the United States, annually 1.7 million patients develop a decubitus ulcer.[2] In skilled care facilities and nursing homes, the prevalence ranged from 2.4 to 23 percent,[3-8] versus 66 among elderly patients admitted for femoral fracture.[9] The treatment of decubitus ulcers in the United States has been estimated to cost $ 6.4 billion in 1994 and $ 8.5 billion in 1997 [10], which is more than the cost of treating patients with AIDS and almost half the amount spent on caring for patients with dementia.[1, 11]

A gold standard for decubitus ulcer treatment is currently lacking, reflected by the broad range of products and interventions for treating these ulcers and by the absence of a superior treatment with a clearly demonstrated efficacy in the database of the Cochrane 'Wound Field' and 'Rehabilitation & Related Therapies Field'. The 'Consensus Decubitus' is only considered a guideline with instructions for prevention, diagnosis, and treatment.[1, 2, 10, 11]

Low Level Laser Therapy (LLLT) has been suggested as a promising treatment option for open wounds. Mester was the first to document the biologic effects of LLLT in case reports [12] and many of his conclusions have subsequently been reproduced in animal studies.[12-18] However, in view of the absence of randomized studies with sufficient large sample sizes in human subjects [19] we performed a prospective, observer blinded, multicenter, randomized clinical trial to assess the efficacy of LLLT in the treatment of decubitus ulcers.

Method

Patients and procedures

The study was carried out in three nursing homes in the Netherlands. Consecutive patients with stage III decubitus ulcers were eligible. Decubitus ulcer stage III was defined as a full-thickness skin defect extending into the subcutaneous layers and adipose tissue.[20, 21] Wounds were limited to stage III decubitus ulcers, because such ulcers are well measurable and laser light penetrates easily in the wound surface. Inclusion was limited to one wound per patient. There were no age restrictions for participation in the study.

Reproducible LLLT treatment was ensured by covering the wound surface area by the physical dimensions of the laser probe completely. Therefore, patients with a wound surface area greater than 30 cm^2 were excluded. Other exlusion criteria were: wounds completely occluded by eschar, because of reduced penetration of laser light in the wound surface area [22]; constant, invariable ulcerations for over one year; diabetic patients with serious metabolic disorders; as well as terminal patients.

After inclusion we recorded the baseline characteristics: age, sex, Norton score, initial wound size, wound duration and decubitus ulcer location. The Norton score is an ordinal risk scoring system for decubitus ulcers containing the items: physical condition, mental condition, activity, mobility, and incontinence. [23] Each item includes a score of 1 (= worst condition) to 4 (= best condition), so individual Norton scores range from 5 to 20 points.

After baseline assessment, all patients were randomly assigned to one of the two treatment protocols; the control group (consensus treatment only), or the experimental group (Low Level Laser Therapy as adjuvant to the consensus treatment). Allocation was by means of a central computerized telephone service. A minimization procedure [24, 25,] concentrating on minimizing imbalance in the distributions of treatment numbers within the various values of each individual possible prognostic factor [24] was performed. The first order minimization factor was 'wound size category' (< 100 mm^2, 100-500 mm^2, and > 500 mm^2); 'treatment center' was the second order factor.

Treatment Regimens

All patients received the prevailing consensus decubitus ulcer treatment as developed and recommended by the (American) National Pressure Ulcer Advisory Panel (NPUAP) [21]; whereas the experimental group had LLLT as adjuvant treatment. Consensus decubitus ulcer treatment was given daily over a period of 6 weeks (max), and involved information and instruction of the patient, wound cleansing, simple moist dressings, and frequent alteration of the patient's position. In the experimental group, LLLT was applied five times a week (except for the weekends). Every two weeks, adherence to the consensus and experimental treatment was checked by examination of medical and nursing records, and by interviews with head nurses and physical therapists using check lists.

LLLT treatments were administered using a 12 microprocessor-controlled infrared GaAs-diode laser probe (Gallium Arsenide) at 904 nm, covering an irradiated area of 12 cm^2 (physical probe dimension 30 cm^2). Total peak power was 12 x 70 Watt in a 830 Hz pulse frequency mode of 150 nsec pulses with an average beam power of 12 x 8 mW and a radiant exposure of 1 J/cm^2, which required an exposure time of 125 sec. The laser probe was applied to the surrounding normal tissue's surface as a so-called contact treatment method, so that the center of the applicator was held just off contact of the wound surface area (distance ≤ 1mm). The beams, with a 2.5˚ angle of divergence, were applied perpendicularly to the tissue to achieve maximal penetration. Equal beam power was guaranteed by using lasers from one production process which were calibrated for all three devices (Combilaser C-501™, Schreuder Medical, Amersfoort, The Netherlands).

An investigator, not involved in the treatment, checked the output of the diode lasers every two months, using an infrared power meter. Before and after each treatment the cluster

probe was cleaned with alcohol (spiritus ketonatus 95%) to prevent cross-infection. All LLLT treatments were given by the same investigator assigned at each of the three facilities. Additional medication which could affect wound healing (e.g. corticosteroids) were not administered and no concurrent adjunctive interventions were initiated during the study. Each included patient (or their representative in case of legal incompetence in psychogeriatric patients) signed an informed consent form. The procedures followed were approved by the Medical Ethics Committees of all institutes and in accordance with the ethical standards on human experimentation of the Helsinki Declaration of 1975, as revised in 1983.

End points

The primary outcome was the absolute (mm^2) and relative (%) wound size reduction at six weeks compared to baseline. In this time frame a meaningful effect on wound healing occurs. [26] Healing (0 mm^2) was scored as a complete wound closure without any dermal residual exudate or inflammation. Every two weeks the wound surface area was registrated in mm^2 based on a full scale (1:1) Polaroid Image Exposure™ (deviation ≤ 1%).
This measurement technique is simple, reliable (Intraclass Correlation Coefficient = 0.99) [27], and easy to accomplish at the bed-side. An independent and trained evaluator outlined the area of these measurements on a transparant wound diagram consisting of a mm^2 grid. The perimeter of the vital borderline of the ulceration was transposed to the transparency and the enclosed area (mm^2) was determined by another investigator, blinded for the clinical details.

The secondary outcomes were the number of patients developing a stage IV ulcer during the six weeks study period, and the median change in Norton scores at six weeks compared to baseline. A stage IV decubitus ulcer is defined as a full-thickness skin loss with extensive destruction, tissue necrosis and damage to muscle, bone, or supporting structures (tendon, joint capsule, etc).

Statistical Analysis

Patients' baseline characteristics were summarized with descriptive statistics. Wound size improvement after treatment was expressed in absolute (mm^2), and in relative terms (%). The patient's relative improvement was calculated as:

1 - follow-up score / baseline score

The differences between absolute and relative wound size improvements were analyzed using the Mann Whitney *U* test. Since the wound sizes were considerably non-normally distributed, we analyzed the primary outcome data after logarithmic transformation of the wound size areas additionally. The difference in mean delta $\log_e$ scores (= $\log_e$ baseline scores - $\log_e$ follow-up scores) between both groups was compared using the unpaired *t*-test. The difference in presence of stage IV decubitus ulcers (secondary outcome) was analyzed using Fisher's exact test.

Table 1

Baseline characteristics of the study group

		Control (*n*=47)	LLLT* (*n*=39)
Nursing home †	I	24 (51%)	20 (51%)
	II	12 (26%)	15 (38%)
	III	11 (23%)	4 (10%)
Age (years)	Mean ± sd	83.5 ± 8.9	81.3 ± 9.6
	Median	85	82
	Range	49-100	49-94
Sex	Male	18	14
	Female	29	25
Norton score	Median	12	11
	Range	5-17	6-18
Wound category ‡	< 100mm^2	17 (36%)	14 (36%)
	100-500mm^2	22 (47%)	20 (51%)
	> 500mm^2	8 (17%)	5 (13%)
Wound surface area (mm^2)	Mean ± sd	350 ± 378	317 ± 396
	Median	232	155
	Range	40-1750	8-1821
Ulcer duration (weeks)	Mean ± sd	3.3 ± 5.1	2.9 ± 4
	Median	2	2
	Range	0.5-30	0.5-22
	Missing ¶	3	3
Location	Gluteal	8	4
	Sacrum / Coccyx	14	14
	Greater Trochantor	1	0
	Medial Femoral Condyle	0	1
	Calcaneus	14	13
	Lateral Malleolus	5	3
	Other	5	4

* LLLT = Low Level Laser Therapy as adjuvant to the standard treatment (control)

† Second order minimization factor

‡ First order minimization factor

¶ Missing data due to transfer from another institution, therefore, the exact ulcer duration is unknown

Based on our pilot study [28] we assumed a mean relative wound size reduction of 50% in the control group compared to a mean relative reduction of 75% in the LLLT group (sd ≈ 40% in both groups). With 40 patients per treatment group this results in 80% power (two sided alpha level of 5%) to detect this difference.

An independent safety committee, the members of which were unaware of the treatment assignements, performed an interim analysis after the inclusion of 40 patients. After blinded, double data entry, all analyses were done with SPSS 9.0, according to the intention-to-treat principle.

Results

Of 105 eligible patients, 19 were excluded before randomization, of which 11 declined to participate and 8 patients were in a terminal state. Randomization began on June 12, 1998; recruitment was completed on December 31, 2000, with follow-up scheduled to continue through to February 14, 2001.

The baseline characteristics of the 86 included patients are shown in Table 1.
The minimization procedure for wound size categories (first order factor) turned out to be successful. However, some imbalance occurred in the patients' places of residence (second order factor) and consequently the number of patients per treatment group (control group *n*=47, LLLT group *n*=39). Mean (±sd) wound size areas were 350 mm^2 (±378), and 317 mm^2 (±396) for the control and LLLT groups, respectively.

During the study period, no protocol violations of standard care and laser treatment were observed. At the end of the treatment period we were unable to assess the wound size area in 13 patients (8 in the control group and 5 in the LLLT group). Of these, four patients (two in both treatment arms) died before the final measurement, one patient was admitted to the hospital, and eight patients developed a stage IV decubitus ulcer, which was considered a secondary outcome in the study protocol. Consequently, the primary outcome assessment concerned 73 patients; 39 in the control group and 34 in the LLLT group.
In the control group 38% (15/39) of the patients showed complete wound healing, whereas in 5% (2/39) the wound size areas had become larger compared to their baseline measurements. In the LLLT group these figures were 53% (18/34) and 18% (6/34), respectively.

Table 2. shows the patients' wound size areas before and after treatment, and the (relative) improvement.

In both groups, the range of wound size reduction during the treatment period varied considerably, from 930 mm^2 improvement to 496 mm^2 deterioration in the control group, and

Table 2

Mean wound size areas before and after treatment, and (relative) improvement

	Control (*n*=39)	LLLT (*n*=34)	*p*-value
Before treatment (mm^2)			
mean ± sd	293 ± 324	248 ± 269	
median	162	140	
range	40 - 1605	11 - 1359	
After treatment (mm^2)			
mean ± sd	116 ± 217	157 ± 380	
median	19	0	
range	0-895	0-1742	
Absolute improvement (mm^2)			
mean ± sd	177 ± 227	91 ± 323	0.50*
median	129	120	
range	-496 - 930	-1007 - 689	
Relative improvement (%)			
mean ± sd	42 ± 213	25 ± 178	0.40*
median	87	100	
range	-1240 - 100	-650 - 100	
Delta $\log_e$ score †			
mean ± sd	2.5 ± 2.2	2.8 ± 2.5	0.64 ‡

* Mann Whitney *U* test

† Delta $\log_e$ score = $\log_e$ baseline score - $\log_e$ follow up score

‡ Unpaired *t*-test

from 689 mm^2 improvement to 1007 mm^2 deterioration in the LLLT group. Mann Whitney *U* tests showed that the differences between the two groups in terms of absolute improvement (p=0.50) and relative improvement (p=0.40) were not significant. Because the wound size areas were non-normally distributed, we additionally analyzed the data after logarithmic transformation of the wound size measurements. No significant difference in $\log_e$ improvement scores between both groups could be demonstrated (unpaired *t*-test: p=0.64).

During the treatment period 11% (5/47) of the patients in the control group, and 8% (3/39) of the patients in the LLLT group developed a stage IV decubitus ulcer (Fisher's exact test: $p = 0.72$). The patients' Norton scores did not change during the treatment period; median Norton scores 12 in the control group and 11 in the LLLT group. No treatment-related adverse effects were reported during this study.

Discussion

In animal studies, [29] 904 nm infrared laser irradiation showed significant greater wound contraction, greater cellular content of granulation tissue, more fibroblast proliferation and better organized fibroblasts at a 700-800 Hz pulse frequency compared to 1200 Hz on surgical skin lesions. Besides that, also regeneration of vein and lymph vessels was reported. [29] The rate of healing was, as in the present study, determined by changes in wound surface area. Furthermore, [13, 30-36] it was found that radiant exposures of ≈ 1 J/cm^2 with Ruby, HeNe, and GaAs-lasers accelerated the wound healing process. Higher radiant exposures (2-4 J/cm^2) in randomized clinical trials on ***human*** subjects showed no effect [19, 28, 37-39], or a doubtful effect [19, 40], on the wound healing process. In our trial we used identical dosage parameters as reported in the successful animal studies.

A number of mechanisms of LLLT has been postulated. This so-called photobiomodulation includes: stimulation of resorption and diffusion [29], activation of the immune system [41], acceleration of the inflammatory phase of wound healing [41], enhanced prostaglandin concentration [42], ATP synthesis [13, 43], collagen synthesis [29, 43], fibroblast proliferation [29, 43], and phagocytosis of macrophages [44], resulting in cellular proliferation and acceleration of the wound healing process. However, anecdotal reports of successful laser treatment of human wounds are plentiful, but controlled human studies scarcely appear in the literature. To date, four randomized clinical trials studying the efficacy of LLLT on wound healing in human subjects have been published. [19, 37- 40] Only one of these studies claimed a statistically significant effect in favour of LLLT. [40] The studies were remarkably different with respect to the type of patients included, the way outcomes were measured and the way LLLT was administered. The validity of their results is jeopardized by very small sample sizes, insufficient blinding of outcome assessment, dissimilarities of prognosis of groups at baseline, withdrawal from treatment and selective drop-out, many co-interventions and missing data. [19] Therefore, doubt persists about the efficacy of LLLT on the promotion of wound healing in human.

Our study did not reveal efficacy of LLLT in stage III decubitus ulcers. We paid specific attention to group size, prognostic comparability at baseline level, and observer blinding. Furthermore, explicit details were given about the laser parameters. An independant party checked for adequate dosimetric output of the laser device before, during and after the trial. Contrary to our pilot study [28], we observed a substantial variability of the wound sizes measured. In the study wound size areas not only improved, but also deteriorated in some patients. Therefore, the question raised whether the number of patients was large enough to detect a possible clinically relevant treatment effect in favour of LLLT. We think, however, that this was the case, since log-transformation of the primary outcome data also revealed non-significant treatment results. With respect to the slight imbalance in the number of patients per nursing home, post-hoc analysis did not show a pattern of different treatment

outcome in relation to the patients' residence.

In this study we did not assess some factors which have been proposed [46] to be of influence on wound healing, such as chronic stress, environmental temperature, concentrations of local (growth) factors, hypovolaemia, blood viscosity, and mechanical stress on the wound. With respect to these factors we depended on the randomization procedure.
The overpresentation of women (63%) in the study group typically reflects a nursing home population. Since decubitus ulcers are not known as sex related, this item is of no consequence with respect to the outcome of the study.

A wide variety of topical applications has been reported to aid healing of decubitus ulcers. In most cases the reports have been anecdotal. The rationale behind the respective treatments has been unclear, in some cases contradictory, and some treatments seem frankly eccentric. [47] Insurance companies, policy makers, physicians, and clinical epidemiologists increasingly require documentation of effectiveness of treatment. In our trial, we found no evidence that justifies using Low Level Laser Therapy as an adjuvant to the consensus decubitus ulcer treatment.

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Chapter 6

Wound Healing in Cell Studies and Animal Model Experiments by Low Level Laser Therapy; were Clinical Studies justified ?

A Systematic Review

Cees Lucas, RPt[1]; Linda J. Criens-Poublon, RPt[2];
Chris T. Cockrell, RPt[2]; Rob J. de Haan, PhD[3]

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[1] Department of Research and Innovation in Health Sciences, University of Professional Education: Hogeschool van Amsterdam, The Netherlands.
[2] Assigned as Student Assistent, Institute of Physical Therapy, University of Professional Education: Hogeschool van Amsterdam, The Netherlands.
[3] Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, University of Amsterdam, The Netherlands.

Abstract

Background Based on results of cell studies and animal experiments, clinical trials with Low Level Laser Therapy (LLLT) were performed, which finally did not demonstrate a benificial effect on outcome of wound healing. The aim of this study was to investigate whether the evidence from cell studies and animal experiments with respect to wound healing was unequivocally in favour of LLLT, which would imply that these models might be adequate to predict treatment response in patients, or that the data of cell studies and animal experiments were inconclusive, which would mean that the clinical trials were based on insufficient evidence.

Methods We performed a systematic review of cell studies and animal experiments with LLLT on wound healing. Manuscripts were identified by searching MEDLINE, EMBASE, and SPIE (the International Society for Optical Engineering).
We assessed whether studies showed a beneficial effect of active treatment or not. The effect size was expressed in standardized mean difference (SMD [the mean outcome measure of the treatment group minus the mean outcome measure of the control group, divided by the pooled standard deviation of these measurements]). In-depth analyses were perfomed on [1] studies in which inflicted wounds on animals were irradiated and evaluated; [2] studies with primary outcome measures on dimensions with direct reference to wound healing (ranging from acceleration of wound closure to epithelialization, but excluding surrogate dimensions with regard to wound healing; in this case: tensile strenght); [3] animal studies with 'true controls'; [4] studies in which animals functioned as their 'own controls' and [5] studies with the highest methodological quality score.

Results The 36 included studies contained 49 outcome parameters of which 30 reported a positive effect of laser irradiation and 19 did not. Eleven studies presented exact data about the effect of active treatment and controls.
The pooled effect size (SMD) over 22 outcome measures of these studies was -1.05 (95% CI: -1.67 to -0.43) in favour of LLLT. Methodological quality of the studies was poor. In-depth analysis of studies showed no significant pooled effect size in studies with highest methodological quality scores [0.06 (95% CI: -0.42 to 0.53)].

Conclusion Summarizing the data of cell studies and animal experiments, reviewed in this manuscript, these studies failed to show unequivocal evidence to substantiate the decision for trials with LLLT in large number of patients.

In fact, there were no differences between the results of these experiments and clinical studies. Remarkably, we found that (almost from the introduction on) animal experiments and clinical studies that address the biological effects of LLLT on wound healing, ran simultaneously, rather than in sequence. We conclude that this type of phototherapy should not be considered a valuable (adjuvant) treatment for this selected, generally therapy-refractory condition in human.

Introduction

The current interest in the wound healing effects of low levels of laser light irradiation and energy stems from the work of the Hungarian surgeon Endre Mester. [1-5] In the past, the terms 'photobioactivation' [6] and 'biostimulation' were frequently used based on the stimulatory effects of this type of laser irradiation, later replaced by 'biomodulation', because inhibitory effects were noted as well. [7] Laser (***L***ight ***A***mplification by ***S***timulated ***E***mission of ***R***adiation) is produced by instruments that emit monochromatic, coherent and collimated light within the red and infrared spectra. A variety of terms have been used to describe this treatment modality. In this paper we have adopted the term Low Level Laser Therapy (LLLT). [8]

Originally, Mester used the blue-green lines of an Argon laser at 488 and 515 nm. Subsequently Helium-Neon (HeNe) laser-emitting red light at a wavelength of 632.8 nm was introduced, now frequently replaced by cheaper more intense, but partially incoherent, diodes with wavelengths between 660-950 nm.

Experimental treatment in patients started in the mid seventies because of reported positive results of irradiation with LLLT in cell studies and animal experiments. However, only four human studies were randomized clinical trials. [9-12] Three of these studies [9-11] failed to confirm the beneficial effect of LLLT, the positive trend of the fourth study [12] was flawed by many co-interventions and a poor methodological quality. In two meta-analyses of clinical studies no statistically significant beneficial effect was found on skin disorders [13] and wound healing [14] respectively. Similarly, discrepancy between initial success in cell and animal studies and subsequent lack of effect in human applications have been reported using LLLT for treatment of acute lateral ankle sprains. [15]

In The Netherlands, a survey among 237 nursing home physicians, 113 dermatologists, and 164 supervisory nurses in nursing homes, rating 30 treatments for stage III decubitus ulcers by scoring their effectiveness, reveiled that LLLT was believed to be ***not*** or ***hardly*** effective. [16] In Northern Ireland, however, a study reported that 65% of physiotherapists surveyed identified wound healing as most popular indication for LLLT. Patients there were quoted as expecting better results from LLLT, calling it the 'miracle cure' or the 'magic treatment'. [17]

In the United States, Low Level Laser Therapy has been well received [18], although its introduction has also been surrounded by controversy. [19, 20] In part, this was due to the paucity of well-designed studies that showed a clinical effect supporting the use of low-energy laser biostimulation. A substantial amount of the research was originally done in Eastern Europe [1-5] and Russia, [21-23] frequently published in non-peer-reviewed journals, often lacking accurate documentation of irradiation protocols and appropriate control groups. [24] Additionally, the variety of laser systems and experimental conditions made comparison of results difficult.

Therefore, the US Food and Drug Administration (FDA) has limited the use of low-energy lasers to 'approved experimental use' and LLLT has yet to receive FDA approval for any indication. [25]

At present, LLLT is still controversial, despite numerous publications in mainstream European and North American journals. Many medical scientists and clinical epidemiologists doubt the validity of the claims, categorizing LLLT as a fringe medical technique for which there is no convincing evidence. [7] Nevertheless, a recent literature review concludes that 'this type of phototherapy should be considered a valuable (adjuvant) therapy for selected therapy-refractory conditions such as the impairment of wound healing'. [8]
Such disappointing and conflicting results have raised doubt about interpretation and validity of outcomes of cell studies and animal experiments to predict subsequent outcomes in clinical research.

This study focussed on the question whether the evidence from cell studies and animal experiments were unequivocally in favour of LLLT, which would imply that these models might be adequate to predict treatment response in patients, or that the data of cell studies and animal experiments were inconclusive, which would mean that the clinical trials were based on insufficient evidence.

Methods

Literature search and inclusion criteria

The literature search for this review was restricted to published results of cell studies and animal experiments, which were identified by searching MEDLINE (Pubmed, 1968-2000), EMBASE (1980-2000), and the database of SPIE (the International Society for Optical Engineering) using the search terms laser therapy / treatment, low level laser, LLLT, HeNe, GaAs, GaAlAs, combined with wound healing, macrophages, fibroblasts, and ATP (limited to 'cell' and 'animal'). In addition, all seemingly relevant 'related articles' were screened for meaningful references. All the retrieved article references were further examined for additional publications. Furthermore, abstracts, congress reports, reviews, and handbooks were checked for relevant citations. The search strategy was carried out as an independent double retrieval procedure (by C.C. and L.C.) based on title and abstract.

Studies were included if they fulfilled the following criteria: [1] the study assessed the effect of LLLT on wound healing in cell- or animal experiments; [2] wavelengths studied had to be 632.8 nm (HeNe) or 660-950 nm (GaAs / GaAlAs); [3] publications had to be written in the English, German, French, or Dutch language.
In case of doubt the whole publication was obtained and evaluated. Subsequently, a third reviewer (C.L.) made the final decision.

Data extraction

From the included original studies the following data were extracted: research method (control group, randomization procedure, blinded outcome assessment), sample type (animal species, number of animals, description of wounds, number of wounds, cell type, wound surface area), intervention (laser treatment parameters), outcome measures, authors conclusion (results of laser irradiation), and reviewers notes (drop-out rate, possibility of statistical pooling, methodological / statistical inadequacy, and final methodological score).

Methodological scores

Based on recommendations [26, 27], we adapted an 8-point rating system to assess the methodological quality of the included cell studies and animal experiments. One point was attributed for each of the following characteristics: (1) dose / response relationship investigated; (2) randomized experiment; (3) optimal time window investigated; (4) monitoring on physiological parameters; (5) blinded outcome assessment; (6) assessment of at least two outcome measures; (7) outcome assessment in the acute phase of wound healing (1-10 days); (8) outcome assessment in the chronic phase of wound healing (3-30 days). Points were granted when these items were mentioned in the report of the study.
Studies scoring < 5 points were graded as 'poor methodological quality', studies scoring from 5-6 points were graded as 'moderate methodological quality', and studies scoring 7-8 points as 'good methodological quality'.
Two authors (C.C. and L.C.) indepently assessed the publications, with respect to the eight categories of the methodological quality scores. In case of disagreement, a third reviewer (C.L.) made the final decision.

Statistical analysis

For each study we defined whether a positive (LLLT beneficial) or negative (no difference between active and placebo / control treatment or deleterious effect of LLLT) result was reported. Pooled analysis in depth was only possible in a limited selection of trials, which reported specific data on the impact of LLLT on wound healing (wound size area, acceleration of wound closure, inflammation epithelialization, collagenization, dermal thickness, histamine release, and tensile strenght). This statistical pooling was performed for the last day of intervention under highest radiant exposure (J/cm^2). Per study, and for each outcome parameter reported, we calculated the effect size in terms of standardized mean difference (SMD [the mean outcome measure of the treatment group minus the mean outcome measure of the control group, divided by the pooled standard deviation of these measurements]), and pooled the individual effect sizes accordingly.
In case the pooled data showed to be heterogeneous, we used a random effects model. [28]
If no heterogeneity was demonstrated, we used a fixed effects model. [29] Statistical uncertainty was expressed in 95% confidence intervals (CI).

Subgroup analysis

In view of our observation that the experimental studies were substantially different with reference to the method of investigation, we prospectively planned subgroup analyses. We identified the following subgroups: [1] studies in which inflicted wounds on animals were irradiated and evaluated (AIAE = ***A***nimal ***I***rradiated, ***A***nimal ***E***valuated); [2] studies in which inflicted wounds on animals were irradiated, while their cells were evaluated after excision (AICE = ***A***nimal ***I***rradiated, ***C***ells ***E***valuated); [3] studies in which cell cultures were irradiated and evaluated (CICE = ***C***ells ***I***rradiated, ***C***ells ***E***valuated); [4] studies with primary outcome measures on dimensions with direct reference to wound healing, e.g. acceleration of wound closure, epithelialization, (pro-)collagen production, granulocyte production, and fibroblast proliferation (thus excluding studies with surrogate outcome measures with reference to wound healing [e.g. inflammation, phagocytosis, and tensile strength]); [5] studies in which animals in the experimental group were compared with 'true controls'; [6] studies in which each animal in the experimental group functioned as their own control; and [7] finally, we investigated whether the methodological quality influenced the results of the experiments.

Results

Description of the studies

We identified 33,181 manuscripts. Based on predefined criteria, 36 manuscripts [30-65] fulfilled the inclusion criteria (a list of these excluded studies is available from the author). Many studies were excluded because they described laser use in plastic surgery, pain reduction, and laser detection of blood flow rates. Detailed characteristics of the 36 included studies are listed in Table 1 (page 120-141), in which they are alphabetically ordered by year of publication. [30-65]

With reference to the animal experiments (n=22) a total of 287 animals were treated with low level laser irradiation (107 animals served as 'true' controls, whereas 152 animals functioned as their own control) after induction of skin wounds. In one of these studies the number of animals in the respective treatment arms could not be verified, [30] in four studies [35, 39, 50, 63] the number of animals was not given at all. A total of 22 studies assessed two or more outcome measures [32, 35, 36, 38, 39, 41, 42, 44, 46, 49-55, 57-60, 62, 64,] none of the studies assessed outcome in an impaired wound healing model. Methodological quality of the studies was poor (median 4; mode 4; range 1-7). Only nine studies mentioned randomization [33, 37, 38, 48-50, 55, 61, 64] of animals or wounds and in just two studies [64, 65] the outcome was assessed by a blinded observer.

Outcomes in general terms

The 36 included studies contained 49 outcome parameters of which 30 reported a positive effect of laser irradiation and 19 did not (Table 2). Positive and negative outcomes, split according to type of experimental designs, were: AIAE 11/13, AICE 5/2, and CICE 14/4, respectively.

With respect to the direct wound healing effects 22 outcome parameters showed a positive effect, while 12 were reported to be negative. With regard to surrogate wound healing effects this ratio was 8/7, respectively. Of the 34 outcome parameters in all animal experiments, 19 involved true controls (positive/negative ratio: 15/4), while in 15 outcome parameters animals funtioned as their own controls (positive/negative ratio: 3/12). Regarding the methodological quality scores for positive and negative outcome parameters, the subdivisions good, moderate, and poor were graded 0/4, 8/9, and, 22/6 respectively (Table 2).

Table 2

Number of included studies (n=36), outcome parameters (n=49), and treatment results

		Number of studies	Outcome parameters	Positive outcomes	Negative outcomes
Overall		36	49	30	19
Subgroups:	AIAE	16*	24	11	13
	AICE	6*	7	5	2
	CICE	16*	18	14	4
	Direct wound healing effects †	25	34	22	12
	Derived wound healing effects ‡	11	15	8	7
	True controls	14**	19	15	4
	Own controls	8**	15	3	12
	Good methodological quality ¶	3	4	0	4
	Moderate methodological quality §	10	17	8	9
	Poor methodological quality ¥	23	28	22	6

Abbreviations and Legenda Table 2:

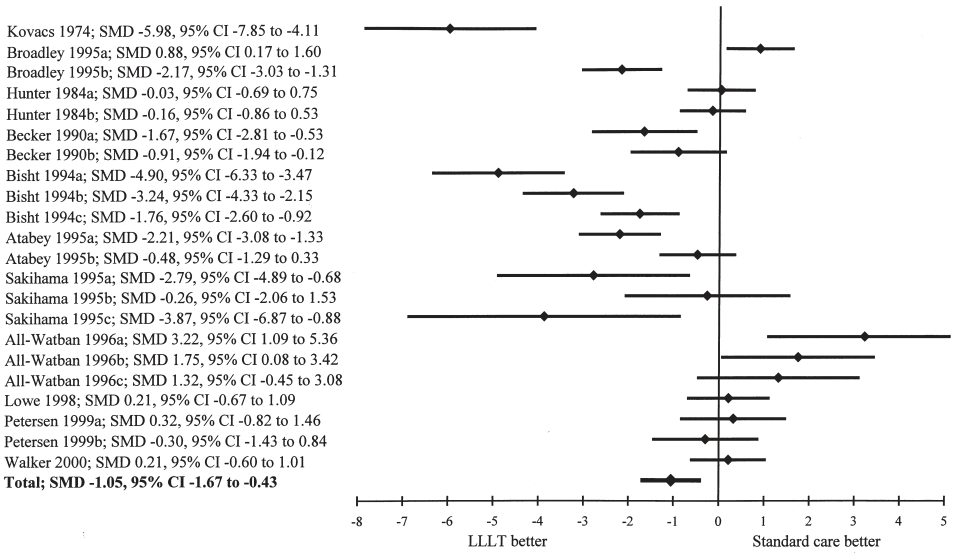
AIAE = Animal Irradiated, Animal Evaluated
AICE = Animal Irradiated, Cells Evaluated
CICE = Cells Irradiated, Cells Evaluated
* Of all included studies (*n*=36), references 51 and 54 scored in two categories (total sum: *n*=38)
** Of all included animal experiments (*n*=22)
† Epithelialization, (pro)collagen production, granulocyte production, fibroblast proliferation, wound closure (days, %, mm^2)
‡ Inflammation, phagocytosis, tensile strength
¶ Good methodological quality 7-8
§ Moderate methodological quality 5-6
¥ Poor methodological quality <5

In-depth analysis

For 25 studies exact outcome measures (e.g. number of animals treated, baseline- and end measurements, and score distribution) were lacking. Consequently the pooled effect size of these studies could not be calculated. In 11 studies [31, 33, 44, 51, 54, 55, 57, 58, 61, 64, 65], exact data about the effect in active treatment and controls was presented. The pooled effect size (SMD) over 22 outcome measures of these studies was -1.05 (95% CI: -1.67 to -0.43) [Figure 1]. In-depth analysis of the 11 studies with respect to the various subgroups could be performed for: [1] studies in which inflicted wounds on animals were irradiated and evaluated (AIAE = ***A***nimal ***I***rradiated, ***A***nimal ***E***valuated); [2] studies with primary outcome measures on dimensions with direct reference to wound healing (ranging from acceleration of wound closure to epithelialization, but excluding surrogate dimensions with regard to wound healing; in this case: tensile strenght); [3] animal studies with 'true controls'; [4] animal studies with 'own controls' and [5] studies with the highest methodological quality score. The pooled

Figure 1

Results of in-depth analysis: Overall effect of LLLT on 22 outcome parameters, presented in 11 studies. SMD = standardized mean difference (effect size)

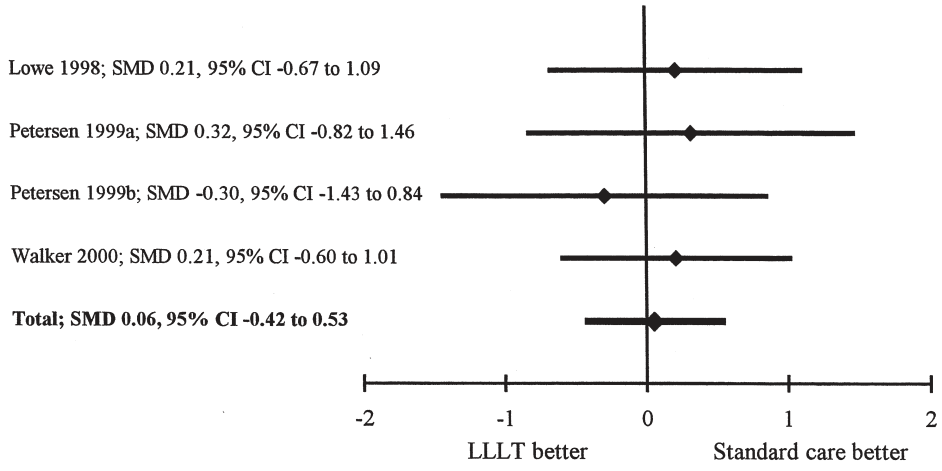


Outcome parameters: wound healing [33, 44, 51, 54, 57, 58, 61, 64, 65] *n*=12, epithelialization [54, 64] *n*=2, granulation tissue [51, 57] *n*=3, collagenization [51] *n*=2, and tensile strength [31, 55] *n*=3

effect sizes (SMD) for these respective subgroups were: AIAE: -0.64 (95% CI: -1.17 to -0.11); Wound Healing: -0.87 (95% CI: -1.44 to -0.31); True Controls: -0.86 (95% CI: -1.28 to -0.44); Own Controls: -0.80 (95% CI: -1.51 to -0.08); and Good Methodological Quality: 0.06 (95% CI: -0.42 to 0.53) [Figure 2]. In all in-depth analyses a random effects model was used.

Figure 2

Results of in-depth analysis: Overall effect of LLLT on 4 outcome parameters in 3 studies [61, 61, 65] with good methodological quality score
SMD = standardized mean difference (effect size)



Discussion

The aim of this study was to investigate whether the evidence from cell studies and animal experiments with respect to wound healing was clearly in favour of LLLT, which would imply that these models might be adequate to predict treatment response in patients, or that the data of cell studies and animal experiments were inconclusive, which would mean that the clinical trials were based on insufficient evidence. The study concentrated on lasers using 632.8 nm (HeNe) and 660-950 nm (GaAs or GaAlAs) wavelengths.

The selected studies showed that the results did not provide an unequivocal answer to the efficacy of treatment with LLLT. Particularly the AIAE-group showed mixed results (Table 2). In contrast, in-depth analysis showed an overall positive pooled effect size. However, this result is probably biased by a substantial influence of four outcome parameters

from three studies with poor methodological quality. [31, 51, 58] Moreover, of all negative studies 47% did not allow for pooling (this equals 38% of all negative outcome parameters) [Table 1]. In 1986, Basford [66] already described the methodological inadequacies of laser studies, including clinical experiments, and posed the question: 'Low-energy laser treatment of pain and wounds: hype, hope, or hokum?' Fifteen years later, with considerably more information at our disposal, we have attempted to answer much the same question. We found that the methodological quality of the studies was poor. In reviewing the published work on LLLT and photobioactivation it has become clear that many of the shortcomings in the literature available are still present, particularly samples that are too small to give a statistically significant result, the lack of appropriate (true) controls, blinded outcome assessment, poor discription of LLLT-parameters and dosimetry, and with inappropriate statistical analyses of the data. The methodological quality of the studies turned out to be associated with the treatment results (Table 2). In-depth analysis showed no significant pooled effect size in studies with highest methodological quality scores [61, 64, 65] (Figure 2).

Results obtained in bilateral inflicted cutaneous wounds, of which only one side irradiated, enhanced significant recovery in both sides compared to the non-irradiated control group. [41] Similar results were obtained in bilateral burns: irradiating one of the burned sites also caused accelerated healing in the non-irradiated site. In the non-irradiated control group, however, animals suffered enhanced necrosis and bilateral gangrene. The statistically significant difference found in the rate of healing of wounds and burns between the non-irradiated side in the irradiated groups and the non-irradiated control groups suggests a systemic effect of low power laser irradiation. [41] In our study, however, we could not confirm the existence of a clear systemic effect. Our pooled analysis showed an effect size (SMD) in 'own controls': -0.80 (95% CI -1.51 to -0.08) in favour of LLLT versus -0.86 (95% CI -1.28 to -0.44) in 'true controls'. Notwithstanding this insignificant difference, studies in which animals function as their own control [33, 34, 51, 54, 55, 57, 64] might be inappropriate to estimate treatment effects precisely.

The pooled analyses were based on comparisons between the mean of the outcome measures in the treatment groups and (own) control groups. Statistically, it would have been better to analyze the difference in delta scores (difference between baseline and outcome). However, this was not possible since the data were not provided in a majority of the articles. The paucity of the data presented was also the reason that we could not adjust the effect size calculated for correlation between wound healing within the same animal. Hence, the standard deviations of the outcome parameters in the own control group may have been overestimated, leading to an underestimate of the effect sizes in this subgroup. On the other hand, we do not think that this has occurred since the variances of the outcome parameters in the control group and own control group were about the same.

We realize that systematic reviews carry hazards such as publication bias and a bias for good quality studies. [67, 68] Evaluating 'old' studies from the early nineteen-seventies enlarges these risks. Since 50% of all experiments was performed more than 10 years ago, we did not approach authors for detailed information about their studies. We considered it to be unlikely that authors either possessed or would remember the exact data required. An exception could be made for the studies with highest methodological quality score. [61, 64, 65] These studies were of recent date (1998-2000) and the authors were able to provide the original raw data for statistical pooling.

Research on LLLT has depended mainly on animal wounds consisting of surgically excised skin. [55] These wound models excluded common problems associated with delayed healing, such as ischaemia, infection, necrotic debris, loss of large amounts of subcutaneous tissue, sinus formation, and induration of surrounding tissue. [69] Therefore, animal wounds that consist of linear (or other artificial) incisions may be inappropriate models for studying laser effects on chronic wounds.

Of the 22 animal experiments 18 articles [30, 31, 34, 37-39, 41, 44, 49-51, 54, 55, 57, 58, 61, 63, 65] demonstrate an effect on wound healing in loose skinned rodents. However, because of their loose skin, wounds in these animals heal predominantly by wound contraction rather than by epithelialization, such as occurs in human. Therefore, any conclusions made from studies on mice, rats, guinea pigs, rabbits, dogs, etc., may not directly be relevant to humans. Due to its similarity, it has been suggested that a better wound healing model for comparison with the human skin is the pig. [8, 66] However, attempts to demonstrate an effect of LLLT on wound healing in a porcine model have not been unequivocally successful. [70-72] This review included one positive [35] and one negative study [33] on pigs. In another positive animal study irradiating pigs, the result was flawed because the laser system contained only one coherent light source among 30 superluminous diodes. [48]

Although the observed benefit of laser therapy has been attributed to light coherence, this concept is not supported by the evidence. [22] Recent cell studies showed no difference in the biological response between coherent laser irradiation and noncoherent light. [22, 42] The skepticism toward the necessity of coherence may additionally be enhanced by the fact that coherence is lost after the scattering events of the incident beam when passing through the first layers of the skin. [21, 22]

Other wound healing studies in animals, with different types of laser systems, have also failed to demonstrate efficacy of LLLT. [73-76] Therefore, it would seem that the balance of current evidence does not indicate a clear beneficial effect of LLLT with respect to wound healing in this type of experiment. [8]

Results of cell studies and animal experiments with LLLT were reported until 1998 and 2000 respectively, more than 30 years after the first clinical studies.[77] It is remarkable that, from the introduction on, these experiments ran a course parallel to clinical studies, since it is reasonable to assume that (to some extent) the clinical studies should be preceded by cell studies and animal experiments.

Summarizing the data of cell studies and animal experiments, reviewed in this manuscript, these studies failed to show unequivocal evidence to substantiate the decision for trials with LLLT in large number of patients. In fact, there were no differences between the results of these experiments and clinical studies. Remarkably, we found that cell and animal experiments and clinical studies that address the biological effects of LLLT on wound healing, ran simultaneously, rather than in sequence. We conclude that this type of phototherapy should not be considered a valuable (adjuvant) treatment for this selected, generally therapy-refractory condition in human.

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Table 1

Detailed description of studies and outcomes

First Author & Year [ref. nr]	Method	Sample Type	Intervention	Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Mester E et al *1973* [30]	Controlled trial AIAE	Wister Rats Inflicted wounds on dorsum Series 1A: n=not given Series 1B: n=20-24 rats Series 1C: n=50 rats Series 2: n=35 rats	Laser treatment: Ruby laser Wave length: 693.4 nm Laser energy: 4x1 J or 2x2 J Average power: - Peak power: - Radiant exposure: 0.55 J/cm^2 Repetition rate: - Pulse duration: 100µs Treatment time: - Angle of divergence: - Spot diameter: - Distance: - Technique: -	Changes in collagen production in second series n=70 wounds *Group* *Treatment* *Control* *%* G1 4395* 3328 33 G2 4300* 2400 45 G3 3020* 2080 32 G4 4800* 3475 28 G5 2750* 2155 22 G6 3800* 2810 27 G7 3400* 2880 16 G8 5390* 4950 9 G9 4170* 3770 10 G10 5392* 4890 10 G11 2795 ns 1430 49 *Groups G1-G11:* Treatment 2x2 J *$p<0.001$ *Groups G1-G6:* Treatment 0 hours after incision *Groups G7-G11:* Treatment 48 hours after incision	Author "cannot agree … with 'lag-phase' in first three days of wound healing process following incision" **Positive Effect** *Note* The generally accepted tri-phasic model of wound healing proposes the proliferation phase to start after 72 hours	No blinding procedure Amount of subdivisions and inconsistent reference to dosage parameters account for cluttered presentation of data References in article conflict with data found in table Numbers of animals in respective treatment arms could not be verified; consequently, these data do not allow for pooling ▶ 4 ◀
Kovács IB et al *1974* [31]	Controlled trial AIAE	Male Sprague-Dawley Rats n=24 Weight: 150 ± 10 gr Inflicted wound on back: 2.5 cm longitudinal slit, sutured over wound edges 1.5 cm total Wound surface: 1 cm Control n=12 Treatment n=12	Laser treatment: HeNe 2x per day; 3, 5, 8 and 12 days respectively first treatment: 4 hours after incision Wave length: 632.8 nm Radiant exposure: - Power: 5 mW Irradiance: - Repetition rate: continuous Pulse duration: n.a. Treatment time: 3 min Angle of divergence: - Spot diameter: - Distance: - Technique: -	Effect on wound healing measured by determining tensile strength Tensile Strength gr *Treatment* *Control* *Day* 120.1 ± 8.8 123.5 ± 7.9 3 361.8 ± 34.8 281.6 ± 25.8 5 644.0 ± 38.8 438.1 ± 17.1 8 748.6 ± 26.2 618.3 ± 16.2 12 *Day* *Difference %* *p-value* 3 - 2.8 5 + 28.5 ns 8 + 47.0 *$p<0.001$* 12 + 26.2 ns	The effect of laser beam on tensile strength is most marked during proliferative vascular activity The laser beam promotes the stimulation of fibroblasts **Positive Effect**	No blinding procedure Data do allow for pooling ▶ 2 ◀

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Mester E et al *1978* [32]	Controlled trial CICE	Human lymphocyte cells Placebo 1 n=500 Placebo 2 n=500 Treatment n=500	Laser treatment Wave length Total Energy Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe Placebo 1: unpolarized monochromatic light Placebo 2: polarized monochromatic light 632.8 nm (placebo 1+2 same wavelength) 4J 50 mW - - continuous n.a. - - - - -	B-Lymphocytes Placebo 1: 0.4% decrease Placebo 2: 30% decrease Treatment: 40 % decrease T-Lymphocytes Placebo 1: 0.8% decrease Placebo 2: 45% decrease Treatment: 50% decrease	HeNe performs an immune surpressor effect Using incoherent light of the same wavelength, effects only occur (80% compared to Laser) if the light is linear polarized **Positive Effect**	No blinding procedure No true control group and no standard deviation given; consequently, data do not allow for pooling ▶ 3 ◀
Hunter J et al *1984* [33]	Randomized trial AIAE	Unpigmented domestic Pigs n=2 weight: 30 kg Partial thickness wounds n=62 Wound surface: 2 cm^2 Control n=35 Treatment n=27 Each animal in the experimental group functioned as their own control	Laser treatment Wave length Radiant exposure Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe 1x per day 632.8 nm 0.96 J/cm^2 64 mW - 64 mW/cm^2 continuous n.a. 15 sec/cm^2 30 ° 1 cm 1 cm scanning	Pig 1 Treatment n=15 Control n=15 Mean % of unhealed wound Pig 1 *Treatment* *Control* *Day* 61.1± 10.6 59.4 ± 14.9 4 55.7 ± 13.6 55.25 ± 15.4 15 day 4 p=ns day 15 p=ns Pig 2 Treatment n=16 Control n=16 Mean % of unhealed wound Pig 2 *Treatment* *Control* *Day* 86.2 ± 9.4 95.1 ± 10.1 6 19.5 ± 7.8 20.6 ± 5.7 16 day 6 p<0.05 day 16 p=ns	No acceleration on wound closure, nor lasting effect in porcine model **Negative Effect** **Negative Effect**	No blinding procedure Data do allow for pooling ▶ 5 ◀
Cakenbergh J van et al *1986* [34]	Controlled trial AICE	Male Swiss Mice n=30 age: 1 month + 20 days to 3 months 2 inflicted wounds: 5mm lumbar/ para- vertebral Control n= 24 (right wound) Treatment n=24 (left wound) Each animal in the experimental group functioned as their own control	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot irradiation Distance Technique	Infra-red diode ; trial period 10 days 835 nm - - - 2 W/cm^2 350 Hz 30 µsec 10 minutes day 0 ; 2x 20 minutes day 1-4 - 44 mm^2 - -	The evaluation method: Two-tailed *t*-test for correlated samples; (t= 0.5) shows no significant influence of laser treatment on wound healing	No evidence of statistically significant stimulation in healing of open skin wounds in mice **Negative Effect**	20% selective drop-out rate No blinding procedure Number of wounds not given, data do not allow for pooling ▶ 1 ◀

Table 1 continued

Detailed description of studies and outcomes

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Saperia D et al *1986* [35]	Controlled trial AICE	Pigs Inflicted full–thickness wound over 1 cm^2 Control group Placebo treatment Laser treatment n=not given	Laser treatment Wave length Radiant exposure Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe 3x per week for 28 days 632.8 nm 0.6 J/cm^2 1.56 mW - - continuous n.a. 5 min - 0.5 cm 2 cm -	Type I and Type III pro-collagen mRNA measurements at days 10, 17 and 28 Type I pro-collagen Day 10 increase 0 Day 17 increase significant (6.5x) Day 28 increase significant Type III pro-collagen Day 10 increase (2x) Day 17 increase Day 28 increase	Observations suggest enhanced wound healing **Positive Effect**	No blinding procedure No exact outcome measures given; only graphical data presentation Number of wounds not given Data do not allow for pooling ▶ 5 ◀

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Lam TS et al *1987* [36]	Controlled trial CICE	Human skin fibroblasts in 96-well tissue cell culture plates Cell line 1 Cell line 2 Cell line 3 Cell line 4 Cell lines 1, 2, 3 and 4: n=24	Laser treatment Wave length Radiant Exposure Average power (Peak) power Irradiance Repetition rate Pulse duration Treatment time Beam spot Beam Area Distance Technique	HeNe and GaAs 1-2 x per day for 1, 3 or 4 days 632.8 nm ; 904 nm 0.053 J/cm^2 – 1.589 J/cm^2 HeNe 1.94 x 10^{-7} J/cm^2 - 5.84 x 10^{-6} J/cm^2 GaAs - 1.56 mW HeNe ; 0.03 mW GaAs 0.883 mW/cm^2 HeNe 0.222 mW/cm^2 GaAs HeNe: continuous, GaAs: 73 Hz HeNe: n.a., GaAs: 200 nsec 1-30 min 14° (ellipsoid) 1.767 cm^2 HeNe ; 0.135 cm^2 GaAs 1.1 cm HeNe and GaAs -	*HeNe* stimulation of procollagen production was measured based on Cell protein dpm [^{3}H]Hydroxyproline/µg *Control cells* line 1 20.68 ± 1.29 line 2 1.55 ± 0.13 line 3 2.27 ± 0.03 line 4 0.15 ± 0.01 *Laser Treated cells* line 1 22.28 ± 1.19 line 2 5.08 ± 0.04* line 3 6.07 ± 0.06* line 4 5.51 ± 0.22* *GaAs* stimulation of procollagen production was measured based on Cell protein dpm [^{3}H]Hydroxyproline/µg *Control cells* line 1 12.24 ± 0.69 line 2 1.62 ± 0.03 line 3 0.22 ± 0.01 line 4 1.57 ± 0.07 *Laser treated cells* line 1 25.31 ± 1.73 line 2 5.17 ± 0.07* line 3 0.19 ± 0.01* line 4 5.53 ± 0.57* *HeNe* and *GaAs* stimulation of DNA replication was measured based on Cell protein dpm [^{3}H]Thymidine *Control cells:* line 1 24.52 ± 1.5 line 2 21.36 ± 1.66 line 3 26.98 ± 1.68 line 4 26.34 ± 9.86 *Laser treated cells:* line 1 25.6 ± 2.02 line 2 19.12 ± 1.5 line 3 4.96 ± 0.3* line 4 2.17 ± 0.04* *: p<0.01	Laser treated cell lines marked with * showed significant increase of hydroxyproline and thymidine **Positive Effect**	No blinding procedure Exact denominator could not be determined; consequently data do not allow for pooling ▶ 4 ◀

Table 1 continued

Detailed description of studies and outcomes

First Author & Year [ref. nr]	Method	Sample Type	Intervention	Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Longo L et al *1987* [37]	Randomized trial AICE	Male albino Sprague-Dawley Rats n=32 Weight: 200-250 gr 1 cm^2 inflicted wound on the back Control: G1 n=8 Alternative treatment: G2 n=8 Laser treatment: G3 n=8 G4 n=8	Laser treatment: GaAlAs daily for 5 consecutive days Wave length: 904 nm, spiked extra short Radiant exposure: 3 J/cm^2 Average power: - Peak power: 20 W Irradiance: G3 10 mW/cm^2; G4 5 mW/cm^2 Repetition rate: G3 3000 Hz; G4 1500 Hz Pulse duration: 200 nsec Treatment time: G3 5 min ; G4 10 min Angle of divergence: 12° vertical , 7° horizontal Spot diameter: 10 – 15 mm Distance: 3 cm Technique: manual scanning 1 cm/sec	Planimetry of healed wounds (% of control) G2 20.9 ± 3.5% $p<0.05$ G3 4.8 ± 14.7% n.s. G4 64.9 ± 4.8% $p<0.01$	Time regeneration in Laser group advanced Histologic findings demonstrated good epithelial regeneration and fibroblastic proliferation at bottom of wound **Positive Effect**	No blinding procedure No data of control group given Data do not allow for pooling ▶ 4 ◀
Lyons RF et al *1987* [38]	Randomized trial AICE	Hairless Mice n=30 Inflicted wound over 6mm in length on the back, followed by suturing Control n=15 Treatment n=15	Laser treatment: HeNe every other day for 8 weeks Wave length: 632.8 nm Radiant exposure: 1.22 J/cm^2 Average power: 1.56 mW Power: - Irradiance: 4.05 mW/cm^2 Repetition rate: continuous Pulse duration: n.a. Treatment time: 300 sec Angle of divergence: - Beam size: 0.385 cm^2 Distance: 0.5 cm Technique: -	Tensile strength measurement at 1, 2, 3, 4 and 8 weeks was based on load and extension of the wound *Treatment* / *Control* / *Week* 12 kg/cm^2 6 kg/cm^2 1 14 kg/cm^2 4 kg/cm^2 2 15 kg/cm^2 8 kg/cm^2 3 11 kg/cm^2 9 kg/cm^2 4 11.5 kg/cm^2 10 kg/cm^2 8 Statistical significant difference in week 1 and 2; $p<0.001$ Hydroxyproline/mg as a unit of collagen concentrations Collagen concentrations measured in 4mm punch biopsies were significantly increased at 2 and 4 weeks following laser irradiation *Treatment* 16.7 ± 2.1 µg/mg (2wks) 28.5 ±1.7 µg/mg (4wks) *Control* 10.5 ± 0.8 µg/mg (2wks) 12.6 ± 1.5 µg/mg (4wks) At 8 weeks the laser treated scar was less noticeable	Overall tensile strength: **Negative effect** Collagen concentrations: **Positive Effect**	No blinding procedure No standard deviation given; consequently, data do not allow for pooling Exact denominator could not be determined; consequently data do not allow for pooling ▶ 6 ◀

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Ikeuchi S et al *1989* [39]	Controlled trial AICE	Male Hartley Guinea Pigs Weight: ± 300 g Control group n=not given Experimental group n=not given 2 round skin defects of 10 mm diameter each were inflicted on the back Skin specimen taken immediately after irradiation on day 0 3, 5, 7 and 14 in both groups.	Laser treatment Wave length Radiant exposure Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe 1x daily for 4 days; trial period: 14 days 632.8 nm 3.6 J/cm^2 6mW - - continuous n.a. - - - - -	Day 0: Hydroxyproline *Control group* 65.5 ± 2.3 µg/mg *Experimental group* 52.6 ± 2.3 µg/mg p<0.025 Day 3: DNA content *Control group*: 0.7 ± 0.21mg/g *Experimental group*: 1.56 ± 0.42 mg/g p<0.025	Treatment group showed increased protein synthesis and earlier and more regularly formed collagen structures Laser inhibites collagenase type I and promotes synthesis of collagen type III Granulation tissue synthesized earlier **Positive Effect**	No blinding procedure Number of wounds not given, data do not allow for pooling ▶ 4 ◀
Ricevuti G et al *1989* [40]	Controlled trial CICE	Human peripheral blood leucocytes n=12 for all groups	Laser treatment Wave length Radiant exposure Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe 5 times 1/ every 12 hrs 632.8 nm 1, 2, 4 J/cm^2 25 mW (minimum output 20mW) - - continuous n.a. - - - - -	Effects of laser irradiation on membrane surface glycoproteins (immunocompetent cells): *Contr* *1J/cm^2* *2J/cm^2* *4J/cm^2* C1 94± 4 92± 5 92± 4 90± 8 C2 92± 4 93± 4 96± 3 94± 4 C3 92± 3 90± 7 95± 3 93± 5 C4 95± 3 92± 4 90± 8 92± 6 C5 90± 8 92± 4 93± 5 90± 8 C1-5: respective cell lines p=ns	No modification of granulocyte aggregation **Negative Effect**	No blinding procedure Data presentation mostly graphical Outcome measures not complete Exact denominator could not be determined; consequently data do not allow for pooling ▶ 4 ◀

Table 1 continued

Detailed description of studies and outcomes

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Rochkind S et al *1989* [41]	Controlled trial AIAE	Sprague Rats Weight: 300 g. *Wound experiment:* Control n=10 Treatment n=10 Bilaterally inflicted on the back: 7x1 cm skin flaps removed. *Burn experiment:* Control n=10 Treatment n=10 Hot water burn of hind legs	Laser treatment Wave length Radiant exposure Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot size Distance Technique	HeNe daily for 21 days 632.8 nm 7.6 J/cm^2 (wounds) 10 J/cm^2 (burns) 16 mW - - continuous n.a. 7 min - 3 mm^2 1 cm -	*Complete wound closure:* Treatment group (irradiated rats) Irradiated wounds 100% (n=10) Non-irradiated wounds 80% (n=10) (contra-lateral side) Control group (non-irradiated rats) Non irradiated wounds:0% (n=20)* $p<0.01$ *Wound healing:* Treatment group (irradiated rats) Irradiated burns 70% (n=10) Non-irradiated burns 70% (n=10) Control group (non-irradiated rats) Non irradiated burns: 0% (n=20) $p<0.01$ * at end point these wounds dimished to half the original size	LLLT accelerates morphological recovery of severely injured tissue **Positive Effect** **Positive Effect**	No blinding procedure Outcome measures given in terms of percentage, consequently data do not allow for pooling ▶ 5 ◀

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Shiroto C et al *1989* [42]	Controlled trial CICE	Blood Samples from 9 healthy male humans Age: 20 years 4 aliquots, each aliquot assigned to a 96 well-plate Experimental plate was divided in 4 sections of 24 wells Each well received different treatment time	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	GaAlAs 830 nm 904 nm - A: 830 nm/ 60 mW B: 830 nm/100mW C: 904 nm/ 3 mW 10W at 904 nm - A: continuous B: continuous C: 1024 Hz - 30, 60, 90 and 120 sec - - 5 mm -	Phagocytosis as measured by oxygen release from the neutrophil; the oxygen reacts with luminol Maximum Luminescence intensity: *Control*: 774.7 ± 149.6 mV *A* 30: 773.8 ± 151.3 ns 60: 789.2 ± 149.6 ns 90: 791.3 ± 148.9 ns 120: 806.0 ± 149.2 $p<0.05$ *B* 30: 776.7 ± 149.5 ns 60: 758.3 ± 158.4 ns 90: 752.6 ± 144.1 ns 120: 758.6 ± 155.2 ns *C* 30: 756.0 ± 128.8 ns 60: 756.0 ± 157.5 ns 90: 754.5 ± 145.2 ns 120: 773.3 ± 160.5 ns During phagocytosis reactivated oxygen is released in volumes proportionate to the strength of the phagocytic reaction time to reach this maximum intensity: *Control:* 21.28 ± 1.57 minutes *A* 30: 19.65 ± 1.24 $p<0.01$ 60: 20.17 ± 1.58 $p<0.05$ 90: 20.54 ± 1.57 ns 120: 20.54 ± 1.46 ns *B* 30: 21.01 ± 1.95 ns 60: 21.10 ± 1.52 ns 90: 21.87 ± 2.06 ns 120: 21.99 ± 1.55 $p<0.05$ *C* 30: 19.19 ± 1.09 $p<0.01$ 60: 19.55 ± 1.56 $p<0.01$ 90: 19.92 ± 0.98 $p<0.05$ 120: 19.55 ± 1.79 $p<0.05$	GaAlAs at 830 nm, 60 mW continuous wavelength significantly altered the maximum level of phagocytic activity and the time to reach maximum level of activity 830 nm at 100 mW retarded both the maximum level of activity and the time to reach maximum activity 904 nm at 10W/ 1024 Hz slightly retarded the level of activity, but shortened the time to reach maximum activity **Positive Effect**	No blinding procedure Exact denominator could not be determined; consequently data do not allow for pooling ▶ 4 ◀

Table 1 continued

Detailed description of studies and outcomes

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Young S et al *1989* [43]	Controlled trial CICE	U-937 Cell line (macrophage-like) Control n=16 wells Placebo n=16 wells G1 n=16 wells G2 n=16 wells G3 n=16 wells G4 n=16 wells Fibroblast Cultures (isolated from Swiss 3T3k mouse kidney) 9ml of medium plus cells	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot size Distance Technique	GaAlAs for 132 hrs max. *Non coherent* *Coherent* G1 660nm G2 820nm G3 870nm G4 880nm 2.4 J/cm^2 15 mW - 120 mW/cm^2 5000 Hz 18 µsec 20 sec 10° (non coherent) 23° (coherent) 0.125cm^2 1 cm -	Fibrobast baseline count n=1.000 for all wavelengths *60 hrs after plating* Placebo 4.800 ± 197 660nm 12.400 ± 121* 820nm 6.800 ± 366* 870nm 6.300 ± 372* 880nm 5.950 ± 266* *84 hours after plating* Placebo 6.300 ± 145 660nm 12.400 ± 236* 820nm 11.000 ± 275* 870nm 9.150 ± 151* 880nm 6.200 ± 236 ns *108 hours after plating* Placebo 7.000 ± 114 660nm 13.400 ± 291* 820nm 12.400 ± 360* 870nm 9.250 ± 305* 880nm 6.200 ± 119* *134 hours after plating* Placebo 9.250 ± 227 660nm 12.500 ± 284* 820nm 12.300 ± 424* 870nm 9.900 ± 176 ns 880nm 6.900 ± 119* * p<0.001	660nm, 820nm, 870nm and 880nm irradiation stimulate fibroblast proliferation 60, 84, 108 and 132 hours after plating **Positive Effect**	No blinding procedure Exact denominator could not be determined; consequently data do not allow for pooling ▶ 5 ◀
Becker J et al *1990* [44]	Controlled trial AIAE	Rats n=16 Control n=8 Treatment n=8 3cm long inflicted skin wound on abdominal wall followed by suturing	Laser treatment Wave length Radiant exposure Average power (Peak) power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe + GaAs treatment group; day 1-2-3-5-8 632.8 nm + 904 nm - HeNe: 5 mW GaAs: 30W - - HeNe: continuous, GaAs: not given HeNe: n.a., GaAs: 160 nsec 10 min. - - 5 mm -	Evaluation by photographical registration on day 1-2-3-5-8 and 15 based on an ordinal 4-point rating scale *Wound healing process day 3* Laser Treatment: 1.68 ± 0.32 Control Group: 2.41 ± 0.53 p=0.027 *Inflammation day 3* Laser Treatment: 1.90 ± 0.40 Control Group: 2.26 ± 0.39 p=ns	Soft laser treatment will improve wound healing in rats, when applied the first few days post surgery **Positive Effect** **Negative Effect**	No blinding procedure Inappropriate statistical procedure; parametric statistics on ordinal data Outcome measures only given for day 3 Data do allow for pooling ▶ 4 ◀

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Bolton P et al *1991* [45]	Controlled trial CICE	U-937 Cell line (macrophages) and 3T3 fibroblast mono-layers Control: G1 Treatment: G2 2.4 J/cm^2 G3 7.2 J/cm^2 Cell count: n=1000 for all groups; viability ranged from 94-97% (fall in cell numbers ns)	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot size Distance Technique	4 days GaAlAS 2 probes 820 nm coherent, polarised light 2.4 J/cm^2 or 7.2 J/cm^2 50 mW, 100mW - 400 mW/cm^2 or 800 mW/ cm^2 5000 Hz 180 µsec *2.4 J/cm^2:* 6 sec (400 mW/cm^2) 3 sec (800 mW/cm^2) *7.2 J/cm^2:* 18 sec (400 MW/cm^2), 9 sec (800mW/cm^2 23 ° parallel to junction plane, 16 ° perpendicular to junction plane 0.125 cm^2 1cm -	Fibroblast baseline count n=1.000 for all groups *2.4 J/cm^2* Days Control 400 800 mW/cm^2 mW/cm^2 0 1000 1000 1000 1 1847±31 2139±44 2482±49 2 2320±116 3353±146 2854±106 3 3722±76 4486±68 6490±209 49428±128 10259±224 13128±250* *7.2 J/cm^2* Days Control 400 800 mW/cm^2 mW/cm^2 0 1000 1000 1000 1 1847±31 2427±84 2622±75 2 2320±116 5544±191 3653±192 3 3722±76 4878±53 4959±90 4 9428±128 12172±179* 8914±247* * p<0.05	Modification of fibroblast proliferation can be obtained by optimising energy density and power density **Positive Effect** (19%) **Negative Effect** (81%)	No blinding procedure Data given in text conflict with data found in tables and graphs Exact outcome measures could not be determined; consequently, data do not allow for pooling ▶ 4 ◀
Breugel v HFI et al *1992* [46]	Controlled trial CICE	Human skin fibroblasts n= 96 wells Control n=6 wells Treatment: G1 n=6 wells G2 n=6 wells G3 n=6 wells G4 n=6 wells	Laser treatment Wave length Radiant exposure Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe 1x per day 632.8 nm 0.06 – 12 J/cm^2 Treatment 1: G1: 0.55 mW G2: 1.24 mW G3: 2.91 mW G4: 5.98 mW Treatment 2: 6.8 mW 1.53 – 21.4 W/cm^2 (irradiated area 0.28cm^2) continuous n.a. Treatment 1: G1: 330 sec G2: 145 sec G3: 60 sec G4: 30sec Treatment 2: 0.5, 1 , 2, 5, 10 min - 0.8 mm (expanded to 3.5 mm) - -	24 % increase in cell proliferation in G2 compared to control group (p<0.05) 20 % increase in cell proliferation in G3 compared to control group (p<0.0002) 34 % increase in Collagen I production in G3 compared to control group (p<0.05) Increase in cell proliferation at powers of G1, G2, G3 with an irradiation time of 30 sec (p<0.0002)	Irradiation of fibroblasts by HeNe laser light may be considered as a trigger for changes in the cell metabolism **Positive Effect**	No blinding procedure No data of control group given; consequently, data do not allow for pooling ▶ 4 ◀

Table 1 continued

Detailed description of studies and outcomes

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Lubart R et al *1992* [47]	Controlled trial CICE	NIH/3T3 Fibroblastic Cell line	Laser treatment Wave length Radiant exposure Average power (Peak) power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe and GaAlAs irradiation 2 days after seeding 632.8 nm 780 nm (diode laser) 0.1- 90 J/cm^2 35 mW or 10 mW - 2 mW/cm^2 HeNe: continuous, GaAlAs: not given HeNe: n.a., GaAlAs: not given - - - - -	Effect of laser irradiation at 632.8 nm on NIH fibroblastic cells: *Dose Cell Mitoses p-value* 0 46 9 43 0.55 (ns) 15 83 0.001 30 56 0.04 60 38 0.04 90 37 0.01 Effect of 780 nm diode laser on NIH fibroblastic cells: *Dose Cell Mitoses p-value* 0 54 7 86 0.0001 18 64 0.02 36 52 0.8 (ns) 72 45 0.05	Laser irradiation shows a significant increase in the number of mitoses in the NIH/3T3 fibroblastic cells in comparison with their respective non-irradiated controls **Positive Effect**	No blinding procedure No data of control group given; consequently, data do not allow for pooling ▶ 3 ◀
Rezvani M et al *1992* [48]	Randomized trial AIAE	Large white female pigs n=16 Age: 12 weeks Weight: 20-25 kg 6 Inflicted, X-ray damaged wounds of 4x4 cm on flank with 4cm separation G1 2 pigs G2 2 pigs G3 3 pigs G4 6 pigs G5 3 pigs	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	GaAlAs multidiode cluster probe 3x per week; from 4–16 weeks or 6–16 weeks after irradiation 660 nm (10 diodes), 820 nm (1 diode), 880 nm (10 diodes), 950 nm (10 diodes) 820 nm diode: coherent light source 660, 880, 950 nm diodes: superluminous 0.22, 0.54, 1.08, 2.16, 4.32 and 10.8 J/cm^2 15 mW (± 20%) - 120 mW/cm^2 2.5 Hz or 5000 Hz 320 ms at 2.5 Hz 160 µsec at 5000Hz 0.2, 0.5, 1, 2, 4 or 10 min - 4 mm per diode 1 cm -	In the 5000 Hz frequency mode, in a time frame from 6-16 weeks at 0.54 J/cm^2 during 30 sec 1.08 J/cm^2 during 60sec 4.32 J/cm^2 during 4 min 10.8 J/cm^2 during 10 min the proportion of wounds developing necrosis was: Treated 15/29 (52%) Expected 29/29 (100%) p=0.001	Low level light treatment can ameliorate the development of late X-ray damage to the skin **Positive Effect**	No blinding procedure No data of control group given; consequently, data do not allow for pooling ▶ 4 ◀

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Karu T et al *1993* [49]	Randomized trial CICE	A/Sn Male Mice Series 1 Age: 8-9 months Series 2: Age: 1.5-2 months Spleen Cells 4x 10^6 cells/ml n=96 wells Well diameter:5 mm 100 µl of cell suspension	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate/ Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	GaAlAs 660 820 870 880 940 and 950 nm 1.1x 10^3 J/cm^2 15 mW *Filtered to* 5% at 660nm, 940 and 950 nm = 0.75 mW 8% at 820 nm = 1.20 mW 7% at 870 nm = 1.05 mW 6.8% at 880 nm = 1.02 mW - - 4.5 Hz / 140 msec 9 Hz / 76 msec 18 Hz /38 msec 36 Hz /23 msec 73 Hz /9.6 msec 146 Hz / 5.2 msec 292 Hz /2.8 msec 700 Hz / 1.15 msec 1000 Hz / 0.82 msec 5000 Hz / 0.16 msec - - - - -	Correlation coefficients between effect of irradiation and % of various cells in spleen suspension (chemiluminence change with 820 nm, 292 Hz and 1,100 J/m^2) *1.5-2 Months* *8-9 Months* *All* *Cells* n=13 n=16 n=29 Lymph -0.417 -0.507* -0.590*** Plasm 0.272 0.748** 0.743*** Mono & Macro -0.031 -0.223 -0.131 Myelo & Metam -0.274 0.463 0.507* Neutro 0.341 0.519* 0.650*** Eosino 0.668 0.071 0.179 Others 0.374 0.318 0.177 *$p<0.05$ **$p<0.01$ ***$p<0.001$ Correlation coefficients between the spontaneous chemiluminescence and percentage of various cells in spleen suspension of mice *1.5-2 Months* *8-9 Months* *All* *Cells* n=13 n=16 n=29 Lymph -0.015 -.0.357 -0.448* Plasma 0.407 -0.194 0.044 Mono & Macro 0.153 0.393 0.253 Mye & Metam -0.114 0.270 0.350 Neutro 0.095 0.429 0.558** Eosino 0.004 0.141 0.102 Others -0.166 0.264 0.078 *$p<0.05$ **$p<0.01$ Legenda Lymph : Lymphocytes Plasma : Plasmacytes Mono & Macro: Monocytes and Macrophages Mye & Metam: Myelocytes and Metamyelocytes Neutro: Neutrophils Eosino: Eosinophils	Irradiation provides for a fine tuning of activity of phagocytic cells, enhancing or depressing it Laser irradiation with certain parameters may affect oxydative processes in cells The stimulative effect of laser radiation was most pronounced at increased percentages of neutrophils and plasmacytes in the spleen **Positive Effect** on statistically significant outcomes **Negative Effect** on statistically non-significant outcome measures	No blinding procedure No standard deviation and exact outcome measures given Exact denominator could not be determined; consequently data do not allow for pooling ▶ 4 ◀

Table 1 continued

Detailed description of studies and outcomes

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Al-Watban FAH et al *1994* [50]	Randomized trial AIAE	Sprague-Dawley Rats n=not given Age: G1 27 weeks Weight: 438 ± 39gr Age: G2 <12 weeks Inflicted elliptical full thickness skin wounds Treatment: G1 1.26 cm^2 G2 0.39 cm^2 Control: G3 Divided over 7 subgroups, further divided over 3 categories	Laser treatment Wave length Radiant exposure Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe 3- 5 x per week 632.8 nm G1 25 J/cm^2 G2 7 J/cm^2 5 mW 18 mW 35 mW - 3.7 – 15.9 mW/cm^2 continuous n.a. - - - - -	G1 49% acceleration in wound size reduction 27% acceleration of wound healing time Control n=3 Treatment n=8 G2 54% acceleration in wound size reduction 33% acceleration of wound healing time Dose >7 J/cm^2: $p<0.05$	Dose dependent wound acceleration in time and size **Positive Effect**	Amount of subvisions and inconsistent reference to dosage parameters account for cluttered presentation of data Data given in text conflict with data found in the graphs Numbers of animals in respective treatment arms could not be verified, consequently these data did not allow for pooling ▶ 4 ◀
Bisht D et al *1994* [51]	Controlled trial AIAE AICE	Male Rats n=38 Weight: 300-350 g Two full thickness skin wounds 8 mm bilaterally on dorsum Control n=38 (right wound) Treatment n=38 (left wound) Each animal functioned as their own control	Laser treatment Wave length Radiant exposure Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Beam diameter Distance Technique	HeNe irradiation daily for 17 days 632.8 nm 4 J/cm^2 - - - continuous n.a. 5 min - 2 mm - -	*Wound closure* Control: 14.1 ± 0.86 days Treatment: 10.3 ± 0.68 days $p<0.001$ *Collagenization:* Mean value of hydroxyproline as quantitative measurement on day17: mg/100mg body weight Control: 28.66 ± 1.019 Treatment: 33.66 ± 1.934 $p<0.01$ Collagen formation as qualitative measurement on day 17: Control: 1.8 ± 0.54 Treatment: 2.9 ± 0.89 $p<0.01$ *Thickness of granulation tissue mean in mm* *Day Control Treatment* 3 0.460 ± 0.213 0.536 ± 0.0197 5 0.790 ± 0.030 1.250 ± 0.002 7 0.920 ± 0.023 1.397 ± 0.029 9 1.154 ± 0.316 1.550 ± 0.0332 $p<0.001$ on all days	Low level laser biostimulatory effects show significant differences in leucocytic proliferation, neo-vascularisation and fibroblastic proliferation **Positive Effect** **Positive Effect** **Positive Effect**	No blinding procedure Inappropriate statistical procedure; parametrical statistics on ordinal data Data do allow for pooling ▶ 4 ◀

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Bolognani L et al *1994* [52]	Controlled trial CICE	Embryonal human Cell line; adenylnucleotides and cytoskeletal structures $5x10^4$ cells per well in 24-well dishes	Laser treatment Wave length Radiant exposure Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe 632.8 nm - 5 mW - 0.64 mW/cm^2 continuous n.a. 5, 15, 30 min - 0.8 mm (beam expander 2 cm) - -	Adenyl nucleotides concetrations expressed as nanomoles / 10^6 cells Treatment Control ATP [1] 5.67 ± 0.17 5.73 ± 0.14 ns ATP [2] 4.15 ± 0.05 4.24 ± 0.12 ns ADP 0.86 ± 0.24 1.42 ± 0.15* AMP 1.98 ± 0.17 0.3 ± 0.02* [1] Luminometric measurement [2] Chromatographic measurement * p<0.05	Low power laser treatment can produce modification of adhesion molecules with a remarkable increase in the capacity of the cells to adhere to the substratum ATP synthesis **Negative Effect** ADP and AMP **Positive Effect**	No blinding procedure Exact denominator could not be determined; consequently data do not allow for pooling ▶ 4 ◀
Yu et al *1994* [53]	Controlled trial CICE	BALB/c 3T3 Fibroblast Cells in well clusters of 5x 10^4 cells Fetal bovine heart endothelial cells in well-clusters of $5x10^4$ cells Each group: n=6	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	Argon Dye irradiation after 24 hrs in the culture 660 nm 0 J/cm^2 2.16 J/cm^2 3.24 J/cm^2 - - 9 ± 0.56 mW/cm^2 - - 4 min & 6 min - 3.5 cm - -	*Cell number ($x10^4$)* *Day 1:* Control 4.6 ± 0.4 2.16 J/cm^2 4.4 ± 0.2 3.24 J/cm^2 4.4 ± 0.2 *Day 3:* Control 14.2 ± 1.3 2.16 J/cm^2 28.5 ± 1.6 3.24 J/cm^2 10.2 ± 1.1 *Day 5:* Control 34.7 ± 4.9 2.16 J/cm^2 83.3 ± 7.1 3.24 J/cm^2 48.1 ± 5.6 All days p<0.001 *Concentration of bFGF* in fibroblast conditioned medium: Control 14.58 ± 0.20 2.16 J/cm^2 17.05 ± 0.21 (p<0.05 *vs* Control) 3.24 J/cm^2 14.25 ± 0.49 (p>0.05 *vs* Control)	660 nm stimulates the release of growth factor-like substance from macrophage like cells. Fibroblasts exhibit increased cell proliferation and enhanced production of bFGF when irradiated with 2.16 J/cm^2 laser energy **Positive Effect**	No blinding procedure Exact denominator could not be determined; consequently data do not allow for pooling ▶ 5 ◀

Table 1 continued

Detailed description of studies and outcomes

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Atabey A et al	Controlled trial	White rabbits n=16 Weight:	Laser treatment	HeNe 1x per 24 hours, starting on day 0 until epithelialization	*Mean epithelialization time* n=16 Control : 12 ± 0.12 days	Epithelialization time **Negative Effect**	No blinding procedure
1995 [54]	AIAE	2300 ± 250 gr 9 cm^2 skin graft on left and right flank		G2 irradiation on day 0 G3 irradiation on day 0 and 1 G4 irradiation on day 0, 1 and 2	Treatment : 11 ± 0.63 days *p*=ns		Data do allow for pooling
		G1 Control n=16 G2 Treatment n=16	Wave length Radiant exposure Average power Power	632.8 nm 1.8 J/cm^2 5 mW -	*Mean wound surface area (mm^2) and contraction rates (%) of full thickness wound on day 7* n=12 Baseline: 400 mm^2	Contraction rate **Negative Effect**	
		Rabbits n=12 4 cm^2 full thickness skin wounds, bilaterally on flank	Irradiance Repetition rate Pulse duration Treatment time	- continuous n.a. 15 min	Control: 281 ± 27 66.5 ± 8.75% Treatment: 266 ± 35 70.2 ± 6.75 % *p*=ns		
		G3 Control n=12 G4 Treatment n=12	Angle of divergence Spot diameter Distance	- - -	*Effect of HeNe on in vitro growth of human skin fibroblasts*	Fibroblast proliferation **Positive Effect**	
		Each animal functioned as their own control	Technique	-	Day Group Cells x 10^6 cell/ml 0 Control 3.9 ± 0.6 1 laser 1 flash 4.1 ± 0.8 ns 3 laser 2 flashes 5.75 ± 0.9* 6 laser 3 flashes 5.9 ± 0.95*		
	CICE	In Vitro 4 groups of Human Fibroblasts cells			* $p<0.05$		▶ 5 ◀

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Broadley C et al *1995* [55]	Randomized trial AIAE	Male Sprague-Dawley Rats n=20 Weight: 275-300 gr Two pairs of transverse inflicted wounds on dorsum 3 cm in length each, followed by 4 sutures per wound Control: G1 n=20 Treatment: G2 n=20 G3 n=20 G4 n=20 Additionally, each rat in experimental groups functioned as their own control	Laser treatment Wave length Radiant exposure Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe daily for 12 days G2, G3 and G4 632.8 nm G2: 0.47 J/cm^2, G3: 0.93 J/cm^2, G4: 1.73 J/cm^2 10 mW - 6.67 mW/cm^2 continuous n.a. - - 5.0 mm 32 cm scanning rate: 4.3, 2.1 and 1.2 mm/sec resp	Tensile strengths *fresh* wounds: Control n=15 Mean: 48.3 ± 9.1 p=0.86 Placebo *0.00 J/cm^2* n=16 Mean: 50.7 ± 8.2 p=na G2 *0.47 J/cm^2* n=17 Mean: 41.9 ± 7.1 p=0.48 G3 *0.93 J/cm^2* n=18 Mean: 55.6 ± 11.9 p=0.70 G4 *1.73 J/cm^2* n=18 Mean: 41.5 ± 6.3 p=0.46 Tensile strengths *fixed* wounds: Control n=16 Mean: 57.2 ± 3.0 p=0.98 Placebo *0.00 J/cm^2* n=14 Mean: 57.0 ± 6.0 p=na G2 *0.47 J/cm^2* n=18 Mean: 74.5 ± 6.6 p=0.041 G3 *0.93 J /cm^2* n=16 Mean: 73.0 ± 4.3 p=0.067 G4 *1.73 J/cm^2* n=17 Mean: 69.9 ± 7.6 p=0.15	Insignificant biological effect of HeNe laser in skin repair Complete re-epithelialization of control group and irradiated groups was observed on day 15 fresh wounds at every radiant exposure **Negative Effect** fixed wounds at every radiant exposure **Negative Effect**	No blinding procedure 10-30% drop-out rate Data do allow for pooling ▶ 5 ◀

Table 1 continued

Detailed description of studies and outcomes

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Hrnjak M et al	Controlled trial	Cultured human embryonic	Laser treatment Wave length	HeNe irradiation 1x or 2x in 24 hours 632.8 nm	Baseline cell count/ml x 10^5: 3.35 ± 0.51 *Group A ($X \pm SD\ x10^5$)*	Single HeNe irradiation demonstrates a	No blinding procedure
1995 [56]	CICE	fibroblasts	Radiant exposure	G A1: 0.5 J/cm² G B1: 0.5 J/cm² G A2: 1 J/cm² G B2: 1 J/cm² G A3: 1.5 J/cm² G B3: 1.5 J/cm² G A4: 2 J/cm² G B4: 2 J/cm²	*End Points:* *Delta Scores:* Control: 2.76 ± 0.77 - 0.59 ± 0.66 G A1: 3.80 ± 1.03 0.45 ± 0.81 $p<0.01$ G A2: 3.91 ± 0.85 0.56 ± 0.67 $p<0.02$ G A3: 3.89 ± 0.69 0.54 ± 0.66 $p<0.025$ G A4: 3.85 ± 0.90 0.50 ± 1.28 $p<0.05$ *Group B ($X \pm SD\ x10^5$)* G B1: 3.66 ± 0.69 0.31 ± 0.99 $p<0.025$ G B2: 3.28 ± 0.72 - 0.08 ± 1.06 $p>0.10$ G B3: 4.15 ± 0.84 0.80 ± 0.85 $p>0.001$ G B4: 5.23 ± 1.21 1.88 ± 0.87 $p<0.001$	significant effect on human fibroblast proliferation in cell culture **Positive Effect**	Exact denominator could not be determined; consequently data do not allow for pooling ▶ 3 ◀
		Control Group					
		Group A irradiation: 1x per 24 hrs 2 dishes / subgroup: G A1 G A2 G A3 G A4	Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter	- - - continuous n.a. - - 5.5 cm (telescope expansion)			
		Group B irradiation 2x per 24 hrs 2 dishes / subgroup G B1 G B2 G B3 G B4	Distance Technique	- -			

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Sakihama H et al *1995* [57]	Controlled trial AIAE	G1 Male ICR Mice n=5 weight: 40-50 gr Induced dermititis in right ears Control n=5 Treatment n=3 Each animal in experimental group functioned as their own control G2: Male Wister Rats n=3 weight: 200-210 gr Peritoneal Mast cells (Aliquots of 0.9 ml) Control n=3 Treatment n=3 Each animal in experimental group functioned as their own control	Laser treatment Wave length Radiant exposure Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe 60, 10, 5 and 0 minutes before and 5 min, 6 hrs and 24 hrs after dermatitis inducing agent 632.8 nm G1: 12.2 J/cm^2 G 2: 6.24J/cm^2, 12.48 J/cm^2, 18.72J/cm^2 8.5 mW - - continuous n.a. - - 5 mm - -	G1: Irritated Contact Dermatitis (ICD) irradiated 5 minutes after induction: ETR *control:* 315.0 ± 31.9 ETR *treatment:* 420.6 ± 12.2 ETR=Ear Thickness Ratio p=0.02 Allergic Contact Dermatitis (ACD) irradiated 5 minutes after induction: ETR *control* : 225.8 ± 7.5 ETR *treatment:* 250.8 ± 10.0 p=0.045 G2: Histamine release from peritoneal mast cells: *6.24 J/cm^2* Control: 13.4 ± 0.1 Treatment: 13.0 ± 0.1 *12.64 J/cm^2* Control: 16.6 ± 0.1 Treatment: 15.1± 0.2 *18.72 J/cm^2* Control: 22.6 ± 0.5 Treatment: 23.4 ± 0.7 p=0.045	LLLT has a inhibitory effect on ACD one hour before induction and on histamine release **Negative Effect** at irradiation 5 minutes after induction on ICD and ACD **Negative Effect** **Negative Effect**	No blinding procedure Drop-out rate 40% in G1 Data do allow for pooling ▶ 5 ◀
Al-Watban F et al *1996* [58]	Controlled trial AIAE	Male Sprague-Dawley Rats Age: 27 weeks Weight: 372-488 gr Full thickness elliptical inflicted wound of 0.39 cm^2 *HeNe* Control n=2 Treatment n=6 *780 nm* Control n=2 Treatment n=3 *830 nm* Control n=2 Treatment n=3	Laser treatment Wave length Radiant exposure Average power (Peak) power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe and GaAlAs 3x per week 632.8 nm 780 nm 830 nm HeNe: 5, 10, 20, 30, 40, 60 J/cm^2 GaAlAs: 10, 20, 30 J/cm^2 HeNe: 40mW; 780 nm: 18 mW 830 nm: 36 mW HeNe: continuous, GaAs: not given HeNe: 10.53 mW/cm^2 780 nm: 5.63 mW/cm^2 830 nm: 11.25 mW/cm^2 HeNe: continuous, GaAlAs: not given HeNe: n.a., GaAlAs: not given - - HeNe 3.8 mm expander used GaAlAs 3.2 mm expander used -	*80% Wound healing in days* Control 10.60±0.14 HeNe 8.61±0.78 Control 10.55±0.21 780 nm 8.53±9.40 Control 10.55±0.21 830 nm 8.90±0.50 *All wavelengths:* p<0.05 *Acceleration in* Wave length *Healing days* *Wound size reduction* HeNe 29.24% 49.87% 780 nm 23.21% 42.31% 830 nm 20.37% 39.79%	At appropriate dosimetric parameters LLLT can promote wound healing in rats **Positive Effect**	No blinding procedure Data do allow for pooling ▶ 4 ◀

Table 1 continued

Detailed description of studies and outcomes

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Luza J et al *1996* [59]	Controlled trial CICE	240 Blood Samples from male and female silver grey cross bred rabbits n=10 Weight:3600 ± 450g	Laser treatment Wave length Laser energy Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe 632.8 nm 0.2 J 0.4 J 0.8 J 1.2 J and 2.4 J - - - continuous n.a. 5 sec 10 sec 20 sec 30 sec and 60 sec - - - -	Leucocyte adherence increased ≈ 15.9% at small dosage <1.2 J Leucocyte adherence decreased at 2.4 J after 60 sec of irradiation $p<0.02$ Polymorphoneuclears (PMNs) adherence increased 25.9% at 0.8 J after 20 sec of irradiation $p<0.02$ PMNs adherence decreased at 2.4 J after 60 sec of laser irradiation Lymphocyte adherence not influenced by irradiation Phagocytic activity of monocytes increased 22% at 0.2 J after 5 sec of irradiation $p<0.02$ Phagocytic activity of PMNs increased ± 10.8 % at 0.2 J after 5 sec of irradiation $p<0.05$ Total count of white blood cells decreased $p<0.02$	Adherence of neutrophils is an initial step for phagocytosis At exposure to greater doses phagocytic activity and adherence significantly decreased **Positive Effect**	No blinding procedure No standard deviation given Exact denominator could not be determined; consequently data do not allow for pooling ▶ 4 ◀
Gagliardi S et al *1997* [60]	Controlled trial CICE	Wistar rats Weight: 150-200 gr Liver mitochondria	Laser treatment Wave length Radiant exposure Average power Power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	HeNe 1x irradiation, followed by measurement after 30 sec 632.8 nm 3 J/cm^2 - - 10 mW/cm^2 continuous n.a. 5 min - - - -	*Vmax (nmol/min x mg) n=3* *Control* *Laser* ADPc 14.8 ± 0.8 13.9 ± 1.0 ns ADPa 19.0 ± 1.0 17.5 ± 1.0 ns ADPk 5.0 ± 0.2 4.1 ± 0.2* ATPc 36.2 ± 2.0 46.9 ± 1.8* ATPk 114.0 ± 2.0 125.7 ± 3.5* AMPk 304.3 ± 12.9 406.0 ± 12.5** *Km (μM) n=3* *Control* *Laser* ADPc 7.2 ± 0.8 4.3 ± 0.6* ADPa 7.0 ± 0.3 6.5 ± 0.3 ns ADPk 53.3 ± 3.1 55.0 ± 2.0 ns ATPc 13.7 ± 1.6 27.0 ± 3.0* ATPk 3.4 ± 0.2 3.2 ± 0.2 ns AMPk 25.3 ± 2.5 28.3 ± 2.1 ns * $p<0.05$, ** $p<0.01$	Vmax measured over ADK and ATP carrier, as well as Km measured over ADP/ATP carier **Positive Effect** Vmax measured over ADP carrier and ATP-ase, as well as Km measured over ADK **Negative Effect**	No blinding procedure Exact denominator could not be determined; consequently data do not allow for pooling ▶ 2 ◀

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Lowe A et al *1998* [61]	Randomized trial AIAE	Balb mice n = 50 Age: 10 weeks Mean weight: 26.72 gr Inflicted wound: 49 cm^2 Control: G1 n=10 G2 (X-ray) n=10 Treatment: G3 n=10 G4 n=10 G5 n=10	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Area of Irradiation Distance Technique	GaAlAs 3x per week for 30 days 890 nm (GaAlAs multi diode n=60) G3: 0.18 J/cm^2 G4: 0.54 J/cm^2 G5: 1.45 J/cm^2 - 300 mW (60 diodes) - 270 Hz - Calculation formula given - 22.5 cm^2 - -	Significant delay in Group 5 as compared to G1 by day 16 p=0.0001 Significant delay in G5 as compared to G2, G3 and G4 from day 14-23 $p \leq 0.0205$ Raw data: *Overall wound size area (mm^2) day 16* Treatment 0.04 ± 0.012 Control 0.02 ± 0.006	Wounds in all groups showed complete closure after 30 days No evidence of stimulatory effect on the rate of wound closure, instead 1.45 J/cm^2 showed an inhibitory effect **Negative Effect**	No blinding procedure. No exact outcome measures given; only graphical data presentation Raw data were provided on request and allowed for pooling. ▶ 7 ◀
Webb C et al *1998* [62]	Controlled trial CICE	Human cells from Chinese female Age: 20 years n=32 *Treatment*: Hypertrophic scar-derived fibroblast (HSF) cell line *Control:* normal dermal fibroblast (NDF) cell line	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot size Distance Technique	1 day irradiation treatment with superluminous diode 5 days of post irradiation measurements 660 nm Noncoherent 2.4 J/cm^2 4.0 J/cm^2 17 mW - 0.078 W/cm^2 (measured at 0.6 cm) 5000Hz 160µs 31 sec (2.4 J/cm^2), 52sec (4.0 J/cm^2) 12° 0.217cm^2 (measured at 0.6 cm) - -	Percentage increase in cell numbers at 2.4 J/cm^2 *HSF* *NDF* Day 1 10.7** 12.3** Day 2 12.3** 11.3** Day 3 8.4** 7.7** Day 4 8.2** 5.1* Day 5 5.3* 4.3* Percentage increase in cell numbers at 4.0 J/cm^2 *HSF* *NDF* Day 1 20.8** 10.2** Day 2 15.4** 9.1** Day 3 16.1** 7.1** Day 4 8.9** 4.3* Day 5 5.5* 3.4ns *p<0.05 **p<0.01 n=32 in all groups including their respective controls	660 nm LLLT may accelerate the healing of indolent ulcers through increasing fibroblast cell numbers, therefore red light wave lengths might be inappropriate for treating burn wounds that are susceptible to hypertrophic scarring **Positive Effect**	No blinding procedure No standard deviation given; consequently, data do not allow for pooling ▶ 5 ◀

Table 1 continued

Detailed description of studies and outcomes

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Al-Watban F et al *1999* [63]	Controlled trial AIAE	Sprague-Dawley rats Age: 27 weeks Weight: 375-482 gr Inflicted full thickness skin elliptical wound in gluteal area: 0.39 cm^2 n=not given	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot size Distance Technique	HeNe , Krypton and GaAlAs 3x per week 632 nm 647-670 nm 780 nm 830 nm 10, 20, 30 J/cm^2 HeNe: 40 mW Krypton: 150 mW 780nm: 18 mW 830nm: 36 mW - HeNe: 10.53 mW/cm^2 Krypton: 35.46 mW/cm^2 780 nm: 5.63 mW/cm^2 830 nm: 11.25 mW/cm^2 HeNe: continuous, GaAlAs and Kr not given HeNe: n.a., GaAlAs + Kr: not given - - HeNe: 3.80 cm^2 expander used Krypton: 4.3 cm^2 reflector used GaAlAs: 3.2 cm^2 expander used -	*Acceleration (%) of wound healing:* *HeNe* 10 J/cm^2 20.75 ± 1.06 20 J/cm^2 29.24 ± 0.95 30 J/cm^2 25.47 ± 1.00 $p<0.05$ *Krypton* 10 J/cm^2 6.73 ± 1.51 20 J/cm^2 14.54 ± 2.43 30 J/cm^2 9.68 ± 1.46 $p<0.05$ *780nm* 10 J/cm^2 15.62 ± 1.70 20 J/cm^2 23.21± 1.54 30 J/cm^2 18.47 ± 1.64 $p<0.05$ *830nm* 10 J/cm^2 10.89 ± 1.79 20 J/cm^2 20.37 ± 1.61 30 J/cm^2 15.62 ± 1.70 $p<0.05$	At appropriate dosimetric parameters LLLT can promote wound healing in rats, optimum dose found with HeNe at 20 J/cm^2 **Positive Effect**	No blinding procedure Krypton irradiation has been added as an experimental treatment to the 1996 study from the same author [58]; methods and other samples in this article are identical, however the number of animals has been omitted No data of control group given; consequently, data do not allow for pooling ▶ 4 ◀
Petersen SL et al *1999* [64]	Randomized single blind trial AIAE	Crossbred Horses n=6 age 8-12 years Surgically induced full thickness skin wound 9 cm^2 on dorsal side of both meta-carpophalangeal joints Control n=6 Treatment n=6 Each animal in treatment group functioned as their own control	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot diameter Distance Technique	GaAlAs cluster probe 1x per day for 30 days, starting 24 hrs after surgery 830 nm 2 J/cm^2 30 mW - - - - 66 sec/cm^2 - - - contact treatment using grid technique	No exact outcome measures given in article Raw data: *Wound size area (cm^2)* Treatment 4.36 ± 1.89 Control 4.88 ± 1.61 *Epithelialization* Treatment 1.50 ± 0.54 Control 1.69 ± 0.65	Wound area increase during first 15 days, after this a decrease occured. No significant differences between means of wound size area nor area of epithelialization. **Negative Effect** **Negative Effect**	Only graphical data presentation Raw data were provided on request and allowed for pooling ▶ 7 ◀

First Author & Year [ref. nr]	Method	Sample Type	Intervention		Outcome Measures	Author's Conclusion	Notes / ▶Method. Score◀
Walker MD et al *2000* [65]	Controlled single blind trial AIAE	*Phase 1*: Balb Mice n=36 Age : 10 weeks Weight: 26.88 gr Control: G1 n=12 Treatment: G2 n=12 G3 n=12 49 mm^2 wound inflicted on dorsum *Phase 2*: Balb Mice n=24 G4 Control n=12 G5 Treatment n=12	Laser treatment Wave length Radiant exposure Average power Peak power Irradiance Repetition rate Pulse duration Treatment time Angle of divergence Spot size Distance Technique	GaAlAs 3 x per week 660 nm G2: 0.5 J/cm^2 G3: 1.5 J/cm^2 G5: 4 J/cm^2 15 mW 0.045 W/cm^2 5000 Hz - G2: 14 sec G3: 42 sec G5: 112 sec - 0.336 cm^2 - contact treatment	*Phase 1* LLLT treatment with 0.5 and 1.5 J/cm^2 had no effect on the rate of wound closure, compared to the control group p=0.27 1.5 J/cm^2 appeared to inhibit wound closure between day 2 and day 7 *Phase 2* LLLT treatment with 4 J/cm^2 had no effect on the rate of wound closure, compared to the control group p=0.65 Raw data: *Overall wound size area (mm^2)* Treatment 0.044 ± 0.064 Control 0.033 ± 0.039 All groups achieved complete wound closure by day 30	LLLT had no beneficial effect on the rate of wound closure **Negative Effect**	Only graphical data presentation Raw data were provided on request and allowed for pooling ▶ 7 ◀

***Key to abbreviations*:**

ref.nr=reference number; Method.Score=Methodological Quality Score; AIAE=Animals Irradiated, Animals Evaluated; AICE=Animals Irradiated, Cells Evaluated; CICE=Cells Irradiated, Cells Evaluated; HeNe=Helium Neon; GaAs=Gallium Arsenide; GaAlAs=Gallium Aluminium Arsenide; bFGF=basic Fibroblast Growth Factor; n.a.= not applicable; ns= not significant; mRNA=messenger Rubo Nuclein Acid; gm/cm=grams per centimeter; na=not applicable

NB Radiant Exposure = Energy Density (J/cm^2)
Irradiance = Power Density (mW/cm^2)

Chapter 7

General Discussion

Parts of this chapter are accepted, entitled:
Low Level Laser Therapy in Wound Management:
Questioning the Theoretical and Biological Assumptions

Cees Lucas, RPt[1]; Rob J. de Haan, PhD[2];
Martin J.C. van Gemert, PhD[3]

Accepted: Lasers in Medical Science

[1] Department of Research and Innovation in Health Sciences, University of Professional Education: Hogeschool van Amsterdam, The Netherlands.
[2] Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, University of Amsterdam, The Netherlands.
[3] Laser Center, Academic Medical Center, University of Amsterdam, The Netherlands.

General discussion

Introduction

For the management of open wounds, over the years, clinicians used a number of physical modalities, among which can be listed: whirlpool, electrical stimulation, ultraviolet radiation, shortwave (pulsed electromagnetic energy), ultrasound, and intermittent pneumatic compression. [1, 2] These treatments are considered to enhance wound healing processes, thereby shortening the length of treatment and reducing patient suffering. The modalities are used as adjuvants to standard clinical care, and efficacy of most of them remains to be established in controlled clinical trials. [1] Most physical treatments assume that, by increasing the blood flow in the tissues, one may either prevent a sore developing in an at-risk patient or effect a more rapid healing in an existing sore. The majority of these modalities have generally been predicted on initiating an inflammatory response (e.g. electrical stimulation and ultrasound). Alternatively, astringents have been applied to reduce inflammation (e.g. creams and medication). It is unexplained why producing an inflammation response and suppressing an existing inflammation should both have a beneficial effect on wound healing.

In the recent past, Low Level Laser Therapy (LLLT) for wound management has been suggested as a physical treatment option and has been commonly used in (Eastern) Europe and Russia for approximately three decades. In the United States however, low level lasers for wound healing have only been used for one decade. Convincing evidence about the efficacy has not yet been established, therefore, these lasers were considered an investigational device by the FDA (Food and Drug Administration) in 1984 [3], and are still considered investigational todate. [4, 5]

In this last chapter the results of the LLLT-studies presented, their implications for clinical practice, and future research are discussed. We will particularly focus on the theoretical framework, the postulated mechanisms of action, animal studies, and clinical studies.

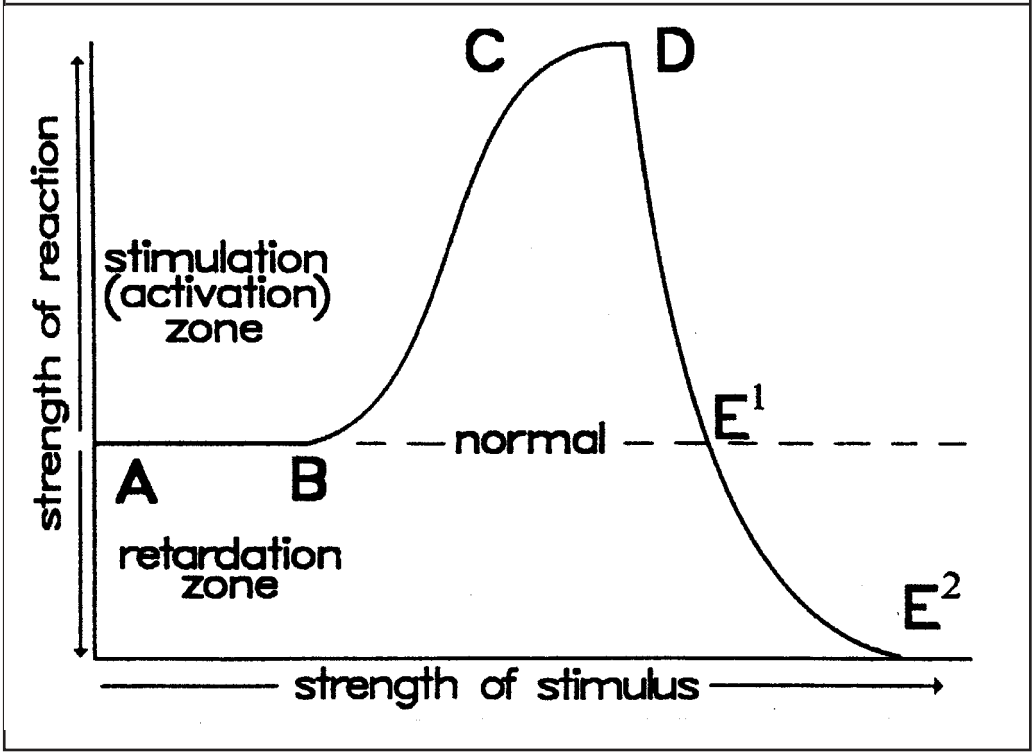
Theoretical framework

In the early 70's, Endre Mester first reported 'photobiostimulation' of wounds as a result of Ruby and Helium-Neon laser irradiation. [6] Since these early reports laser therapy has become a popular treatment choice for a variety of clinicians, including physical therapists, primarily in Europe and the former Soviet Union. Studying the carcinogenic effect of repeated ruby laser radiation, Mester noted that low energy (1 J/cm^2) impulses stimulated hair growth in depilated mice. [6] He noticed that biologic effects of repeated impulses accumulate, and that above a certain value an inhibitory effect was produced. [7, 8] To his opinion, this occurrence corresponded to 'the basic biological law of Arndt-Schulz', and 'was verified in several biologic systems' [9], among others in wounds of mice. [10] Mester was the first to propose the dose-dependend relationship and biologic response, based on this Arndt-Schulz principle

(Figure 1). It theorizes that tissues react to the amount of energy absorbed per time unit. Weak stimuli excite physiologic activity, moderately strong stimuli favour it, strong stimuli retard it and very strong stimuli arrest physiological activity. [11] Many authors duplicated this theory since. [12-15] The claims however, are not based on published laboratory tests, experimental animal studies, or clinical studies.

Figure 1

Graphic representation of the principle of the Arndt-Schulz law



One of the first publications with respect to the Arndt-Schulz law describes the dose-response relationship of cold- and warm water treatment on blood circulation. [16] With regard to this principle, the temperature of the agents seems to play an important role in the observed effects. Low-energy lasers, however, emit power densities (irradiances) that are too low to cause temperature increases beyond 0.5°C in the target tissue. [17] Although it is generally believed that the Arndt-Schulz law provides a useful theoretical basis to explain the varying photobiostimulatory and photobioinhibitory effects, [14] the question raises whether this principle is indeed applicable to low level laser irradiation.

Since the energy density (radiant exposure; J/cm^2) is the most important factor in

determining the tissue reaction, [18] post-hoc, we analyzed part of the data obtained in our systematic review of cell studies and animal experiments with respect to this parameter (Chapter 6). Contrary to theoretical assumptions, we computed substantially higher energy densities in the positive studies compared to the studies with a negative outcome (Table 1). This phenomenon was observed in Helium-Neon (HeNe), as well as in Gallium (Aluminium) Arsenide [Ga(Al)As] laser irradiation and was found in all experimental designs as described in Chapter 6. In one of these subgroups (AIAE; studies in which inflicted wounds on animals were irradiated and evaluated) we could demonstrate that this difference was statistically significant for HeNe irradiation (p=0.03) and Ga(Al)As irradiation (p<0.01).

Table 1

Differences in energy density (radiant exposure; J/cm^2) in relation to experimental outcomes [1], differentiated for subcategories [2] and laser type [3]

Study type	AIAE	AIAE	AIAE	AIAE	AICE	AICE	AICE	AICE	CICE	CICE	CICE	CICE
Laser type	HeNe	HeNe	Ga(Al)As	Ga(Al)As	HeNe	HeNe	Ga(Al)As	Ga(Al)As	HeNe	HeNe	Ga(Al)As	Ga(Al)As
Outcome	+	-	+	-	+	-	+	-	+	-	+	-
Mean ± sd	13.6±8.0 *	6.2±6.7 *	16.3±17.3 †	1.5±1.3 †	2.4±1.7 #	1.22 #	3		6.5±19.8 ‡	2.5±1.3 ‡	13.1±31.2 ¶	3.6±3.2 ¶
Median	11.1	1.8	10	1.5	2.4	1.22	3		1.55	2.5	2	2.4
Mode	10	0.47	10	0.2	0.6	1.22	3		2.4	1	2.4	1.1
Range	4-30	0.5-18.7	0.2-60	0.2-4	0.6-4	0	0		0.5-90	1-4	0.5-90	1.1-7.2
n [4]	12	9	15	7	4	1	1		20	4	8	3

1 + positive study outcome, in favour of LLLT
- negative study outcome, not infavour of LLLT

2 AIAE ***A***nimal ***I***rradiated, ***A***nimal ***E***valuated; studies in which inflicted wounds on animals were irradiated and evaluated
AICE ***A***nimal ***I***rradiated, ***C***ells ***E***valuated; studies in which inflicted wounds on animals were irradiated, while their cells were evaluated after excision
CICE ***C***ells ***I***rradiated, ***C***ells ***E***valuated; studies in which cell cultures were irradiated and evaluated

3 HeNe Helium Neon; Ga(Al)As = Gallium (Aluminium) Arsenide

4 *n* number of different radiant exposures

\# *p*-value could not be calculated because of insufficient number of radiant exposures in the negative subcategory

* p=0.03; † p<0.01; ‡ p=0.33; ¶ p=0.68 (Mann Whitney *U* test)

In conclusion, the effects of low power laser irradiation on wound healing are presumably not attributable to thermal events. Furthermore, evaluating energy densities in relation to experimental outcomes, we could not confirm the existence of the Arndt-Schulz principle.

A variety of different types of laser light sources has been described delivering laser energy at low levels. The HeNe and Ga(Al)As lasers have been used in most of the recent studies (including our clinical studies, Chapter 3 and 5), but the incident energy density, the total dose delivered, and the treatment schedules followed have varied considerably from one study to another. This variability, combined with the fact that different cells and tissues have been used as targets for irradiation, may explain part of the variable and even controversial results reported in cell, animal, and clinical studies.

Postulated mechanisms of action of LLLT

Many of the recent studies deal with the effects of low-energy lasers on cellular metabolism, extracellular matrix production, tissue repair, and immune functions of cells. The stimulation of collagen gene expression and an alteration in the protein synthesis at the transcriptional or posttranscriptional level are two components that have been postulated as a mechanism of action in wound healing by low-energy lasers. [19, 20] These effects can be attributed to direct modulation of regulatory elements within the cells, such as the promoter regions of type I and III collagen genes that have been shown overexpressed after laser irradiation. [21, 22] Similarly, laser radiation may have a direct effect on cell proliferation by affecting the nuclear chromatin structure, which regulates cell proliferation. The effects could also be more indirect, as suggested by the finding of enhanced uptake of ascorbic acid after laser irradiation, this vitamin being a critical cofactor in collagen formation. [23] Furthermore, the effects can be indirectly elicited by paracrine factors, as indicated by the release of fibroblast stimulatory factors from macrophage-like U-937 cells after laser irradiation. [24]

Karu has proposed, but not proven, a unifying hypothesis embracing the various molecular events triggered by laser irradiation. The central theme of her proposal is that components of the respiratory chain are the primary photoacceptors of laser energy. [25] The photosignal transduction and amplification that occur are then determined by the physiologic state of the cell at the time of laser irradiation. If the redox potential in cells is low, the magnitude of the laser effect will be stronger than in cells with higher redox potential. [25] These mechanisms of photosignal transduction have been proposed to involve the absorption of laser energy by enzymes activating the mitochondrial respiratory chain. The resulting changes in this chain alter the redox potential by accelerating electron transfer, which in turn activates the electrical potential of mitochondria and increases the intracellular pool of ATP. These events may lead to an increase in the intracellular hydrogen ion concentration, a necessary component for mitogenic signal transmission in the cells. [25] These events may also alter phenomena that activate the membrane ion transport systems, including the

sodium-potassium pump and the activities of ATPase. Subsequently, the changes in cellular redox potential may then alter proliferation, macromolecular synthesis, and response of cells to immunologic modulator molecules.

As evident from this overview of possible mechanisms of action of low-energy lasers, there is an abundance of yet to be proven hypotheses on the biologic effects.[25, 26] In our opinion, the efficacy of low-energy laser irradiation in the context of certain biologic or cellular functions lacks conclusive scientific evidence and therefore remains doubtful.

Animal studies

Small, loose-skinned rodents such as mice, rats, guinea pigs, rabbits and dogs have been the animals most often used in studies on wound healing. This has been attributed to their relative ease of handling and examination, availability in large numbers, and low death risk upon anaesthesia. Several articles demonstrated an effect on wound healing in loose skin rodents, all possessing a loose elastic skin and a panniculus carnosis, a thin subdermal muscle layer with few deep attachments. These features allow rapid wound healing largely by contraction, while epithelialization is of lesser importance. In tight-skinned mammals, however, contraction also contributes to wound healing, but epithelialization plays a more significant role. The pig is a good example of a tight-skinned mammal with dermis analogous in structure and healing behaviour to human dermis.[27, 28] However, attempts to demonstrate an effect of LLLT on wound healing in a porcine model have not shown to be unequivocally in favour of LLLT.[27, 29-31] In one positive study on wound healing in pigs the result was flawed because the laser system contained only one coherent light source among 30 superluminous diodes.[32]

Some studies mentioned the possibility of systemic effects following treatment with low-power lasers. Kana et al.[33] claimed that argon irradiation induced an increase in collagen synthesis at the site of application as well as at the contralateral side. They attributed this effect to an immunosuppressive influence of LLLT, but there is no direct evidence for its existence. Mester et al.[34] reported that treating one corneal lesion with low-power laser irradiation stimulated the healing of the other nonirradiated injured cornea. He explains this phenomenon by an increase of phagocytic capacity of leucocytes following laser irradiation. Mester's hypothesis is supported by the fast skin wound epithelialization, since the phagocytic activity of leucocytes is known to play an important role in clearing tissue debris.[35] A similar systemic effect has been observed in the peripheral and central nervous system. Low-power laser irradiation applied to crushed injured sciatic nerve in the right leg of rats (in a bilaterally inflicted crush injury) significantly increased the compound action potential in the left nonirradiated leg as well.[35] This systemic effect was also found in the spinal cord segments corresponding to severely injured nerves. The bilateral retrograde degeneration of the motor neurons of the spinal cord expected after bilateral crush injury of the peripheral nerves was

'substantially reduced' in the laser treated group. [35] Finally, the systemic effect has been found in bilateral inflicted cutaneous wounds and bilateral burns. [35]

Our systematic review (Chapter 6) did not show an indisputable existence of a systemic effect. In this study we estimated the pooled effect size (standard mean difference) of LLLT in 'own controls' (-0.80 [95% CI: -1,51 to -0.08]) versus 'true controls' (-0.86 [95% CI: -1.28 to -0.44]). However, the difference found in other studies with respect to the rate of healing of wounds and burns between the non-irradiated side in the irradiated groups and the non-irradiated control groups, [35] indicates that studies in which animals function as their own control might be inappropriate to detect significant differences in wound healing. Consequently, future studies must be carried out using rigorous controls as independent groups, which allow parallel analysis of treated and untreated tissues, and not just tissue harvested from untreated areas of the skin from the same test subject.

Another important consideration is that animals normally used in experiments are relatively young and healthy. These animals have excellent wound healing response. [28] Furthermore, research on LLLT has depended mainly on animal wounds consisting of surgically excised skin. These wound models excluded common problems associated with delayed healing, such as ischaemia, infection, necrotic debris, loss of large amounts of subcutaneous tissue, sinus formation and induration of surrounding tissue. [36] Therefore, animal wounds that consisted of lineair incisions may be inappropriate models for studying laser effects on chronic wounds. Since the results of animal experiments are to be extrapolated for application in human research or clinical practice, it may, therefore, be more valid to examine a healing-impaired model. Evaluating the effects in diabetic, or aged animals, for example, would be more appropriate.

Finally, it should be stressed that our systematic review of cell studies and animal experiments (Chapter 6) shows poor methodological quality. Only the most recent animal studies scored highest rankings on methodological quality, all showing negative effects on wound healing. [43-45]

Clinical studies

In Chapter 2, we described a systematic review of four randomized clinical trials in human subjects. From three of these studies, [37-39] the overall effect size estimate indicates that LLLT had no significant beneficial effect on wound closure (pooled RR=0.76 [95% CL: 0.41 to 1.40]). One negative trial [40] was excluded from this analysis because its outcome measure was defined as time needed to complete wound healing (survival analysis), while the other studies had wound size reduction as an outcome measure. It is noteworthy that the three negative studies [37, 39, 40] had a relatively better methodological quality score. The positive

trend of the remaining study [38] was flawed by many co-interventions. In Chapter 5, we presented the results of our clinical trial in which the effect size was expressed in terms of wound size reduction using our combined measurement method as described in Chapter 4. Here again, we could not demonstrate a favourable effect of LLLT.

Apart from application in the treatment of chronic ulceration, the use of LLLT has also been advocated for the treatment of (acute) postsurgical wounds. [41] However, a recent study to investigate the efficacy of LLLT in the management of uncomplicated postoperative wounds after minor surgery indicated that there were no statistical significant differences between groups for wound closure. [42]

In conclusion, currently no universally accepted theory can explain the mechanism of laser biomodulation. Although a theoretical understanding is not necessary to establish effects, the lack of knowledge complicates the evaluation of conflicting results found in literature. [46, 47] Both animal experiments and clinical studies did not reveal an unequivocal treatment effect of LLLT. Nevertheless, there still seems to be a desire to believe in the efficacy of LLLT in wound healing, suggesting that enthusiastic researchers sometimes seem to loose their critical attitude and start, and continue clinical studies and treatments based on too weak scientific evidence. It is about time that we honestly look at our decision-making process, realizing that it becomes difficult to ethically justify any treatment, especially if the underlying research methodology is so poor, or the published reports so scant, that the results cannot be relied upon.

In Dutch consensus texts concerning decubitus ulcer treatment there is a distinction between treatments which are (expected to be) 'effective', treatments which are 'probably effective', and treatments which are considered 'useless'. [48] For topical applications, ultrasound is categorized as 'probably effective', while ultraviolet radiation and xanithol-nicotinate iontophoresis are classified as being 'useless'. To date, consensus texts do not include LLLT yet, [48, 49] to our opinion it is worth considering to include this treatment in the 'useless' category.

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Epilogue

Epilogue

Evidence based practice can be described as: the conscientious, explicit and judicious use of current best available evidence in making decisions about the care of individual patients, integrating individual clinical expertise with the best available evidence from systematic scientific research. [1]

The concise practice of evidence based medicine comprises five steps: [1] [1] converting the need for information into an answerable question; [2] tracking down the best evidence with which to answer that question; [3] critical appraising the evidence for its validity, impact, and applicability; [4] integrating the critical appraisal with clinical expertise and with the patient's unique biology, values, and circumstances; and [5] evaluating the effectiveness and efficiency in steps 1-4, and seeking ways to improve them both for the next time.

There is little dissent to the principles of evidence based practice and the need for research into current practices has been acknowledged and embraced by many health care practitioners. Moreover, it is satisfying to read the results of a clinical trial which 'proves' the efficacy of a treatment approach that we are currently using in clinical practice. However, what of the evidence that is not supporting some particular practice? Have we been incorrect in using this method all these years, and indeed this may well be the case for certain treatments, or are there limitations to the research?

A factor to appreciate is that the randomized clinical trial is but one method of research, albeit an important one, that contributes to evidence based practice. Other research methodologies are equally important, for example, pre-clinical study designs which investigate questions of mechanisms of action of interventions which cannot always be answered in an experimental design of treatment efficacy. Research methods investigating epidemiological aspects (e.g. long term outcome of injury and disease) also make a valuable contribution to the evidence base. Another area that is a particularly important one, deals with prognostic studies directed towards identifying the patients for whom a certain intervention is relevant.

The need for such research becomes very clear in case treatments are being implemented into patient management schema. There is a burgeoning of clinical practice guidelines, which in themselves, can be helpful in guiding patient management if constructed in a careful and relevant way on the evidence available and implemented using clinical experience and knowledge. However, attempting to treat conditions such as chronic wounds, as reflected in some guidelines, is fraught with difficulties. There may be evidence for a certain treatment approach but its applicability across the spectrum of subgroups and the recognition of responders and non-responders to physical interventions must be carefully evaluated.

The studies presented in this thesis have been unable to demonstrate the effectiveness of LLLT on wound healing unequivocally. This does not necessarily mean that low level laser treatment (LLLT) is incapable of producing the desired wound healing effect. Perhaps, the negative studies merely demonstrate that the selected parameters were not effective. Or, as Altmann and Bland stated 'Absence of evidence is not evidence of absence.' [2] Otherwise, a study, using various dosages has also failed to show any effects on cell metabolism, and hence has not provided corroborative evidence as well. [3] To our opinion, the claim that LLLT has an effect on wound healing must be refuted and before LLLT can be accepted as a useful clinical technique, particularly at a time when healthcare resources are so stretched, the remaining discrepancies will have to be resolved.

As evident from the overview of possible mechanisms of action of low-energy lasers, there is an abundance of hypotheses on the biologic effects. This information is often difficult to interpret, while these effects themselves are described as 'incredible and mysterious' [4] and LLLT is referred to as 'the miracle cure' or 'the magic treatment'. [5] The lack of a clear cut and convincing biological basis that explains the clinical effects induced by LLLT, serves to maintain the conflicts about proper dosage and treatment indications. Skin disorders are thought to react positively to laser irradiation. Therefore laser therapy is being widely used in physiotherapy. [5] A further analysis of the potential effects of LLLT in the wound healing area seems imperative, especially because previous reviews are outdated [6] and/or non-systematic. [6,7]

The value of a literature review depends on the success in obtaining the results of all trials which have been conducted on the issue of interest. It is possible that relevant studies in fora not accessible to us, or in languages incomprehensible to us, were omitted from our systematic reviews. There are also indications that, especially, small clinical trials with negative results are not as easily published as small positive trials. [8] Therefore, publication bias could form a threat to the validity of the results presented in this thesis. In the latter situation however, the overall negative outcome would only have been more conclusive.

Unfortunately, we have not found the cure for decubitus ulcers, neither do we think this will come about on short term. The pressure sore represents a destructive process associated with aging and should not be neglected. With a population that is living longer and is exposed to more accident enhanced immobility, we can expect an increase incidence of decubitus ulcers. One of the main challenges to clinicians and researchers especially in the area of wound healing, will be to predict the responders and non-responders to a certain method of treatment. Some predictors of poor responders are available, but these do not account for many of the patients seen in daily practice.

The management of chronic ulceration and delayed wound healing represents a significant problem for a variety of healthcare professionals. The elderly, those confirmed to

bed, and longterm diabetics often present with sores and ulceration that defy conventional treatment and cause considerable discomfort and suffering for the patient. These wounds often lead to major deterioration in the quality of life and an enormous cost associated with hospitalization. Therefore, there is a need to understand the deficit in the repair process induced by such complications and to develop therapeutic strategies for intervention. Moreover, in this era of 'evidence based medicine', there is a stringent demand to align our clinical practice according to the best available evidence. If we fail to make these decisions rationally and our selection of specific treatments is based on authoritarian advice or our conviction that such therapy ***seems*** to work or ***ought*** to work, not only worthless treatment be applied; sometimes it might be downright harmful. Today's therapy, when solely derived by induction from biologic facts or uncontrolled clinical experience, may become tomorrow's bad joke. Only through exhaustive investigation performed under controlled protocols, will the real benefits of low energy lasers, if any, eventually be demonstrated. Until then, it is recommended to refrain from further use of (infrared) low energy laser irradiation in the treatment of chronic wounds.

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Summary

Summary

The ***Introductory Chapter 1*** of this thesis, defines the four stages of decubitus ulcers and describes the pathophysiological, clinical, and patient related risk factors for developing these ulcers. Furthermore, the background of Low Level Laser Therapy (LLLT) is described as a possible treatment option for chronic wounds.

In ***Chapter 2,*** we present a systematic review summarizing the efficacy of infrared Low Level Laser Therapy on wound healing in human subjects. In order to retrieve randomized clinical trials, we performed computer aided searches of databases MEDLINE, EMBASE, CINAHL, SPIE, and the Cochrane Database) and of bibliographic indexes. Furthermore, congress reports, reviews and handbooks were checked for relevant citations. Subsequently, all retrieved and blinded studies were scored on methodological quality. We found 4 randomized clinical trials that investigated the effects of LLLT versus placebo or any other intervention. Only one trial demonstrated a beneficial effect. Overall, study quality ranged from poor to insufficient.
Of three studies we could perform a meta-analysis. The overall effect size estimate indicates that Low Level Laser Therapy had no significant beneficial effect on wound healing (pooled RR=0.76, 95% CL 0.41 to 1.40). At present, we conclude that there are no scientific arguments for routine application of infrared (904 nm) LLLT on wound healing in patients with decubitus ulcers, venous leg ulcers (ulcus cruris), or other chronic wounds.

Chapter 3 describes a randomized pilot study in four nursing homes.The objectives of this pilot study are: [a] to assess the feasibility of a multicenter trial in a nursing home setting; [b] to investigate whether the type of evaluation method is applicable; [c] to assess the extent of wound size reduction in both treatment arms for an adequate power analysis for a future trial; and [d] to analyze the treatment effect(s) of a gallium aluminium (GaAl) 904 nm cluster laser (consisting of 12 infrared diodes) at a radiant exposure (energy density) of 1 J/cm^2 on tissue repair of full thickness stage III pressure sores.
A total of 20 patients were enrolled into the study, 16 patients were randomized, and four patients were excluded. Treatment was the prevailing consensus decubitus treatment (n=8); one group (n=8) had 904 nm LLLT in addition, five times a week over a period of six weeks. The main outcome measure was the median wound size at six weeks after intervention.
No statistical significant difference was found in wound size ***between*** the two groups (Mann Whitney U test; p=0.47). The median wound size reduction compared to baseline was 83% in the LLLT group and 95% in the control group. There was a significant wound decrease ***within*** treatment arms (Friedman Two-way Analysis p<0.001).
It was concluded that a multicenter study is feasible in nursing homes, whereas the evaluation methods turned out to be easy and accurate. A large scale clinical trial is needed to demonstrate the efficacy of LLLT. In preparation of such a trial, we calculated that a sample size of at least 74 patients (37 subjects per treatment arm) would be necessary to detect an

average improvement of log 0.3 delta in favour of the experimental group with a two-tailed level of significance (alpha) of 0.05 and a power of 0.80.

In ***Chapter 4,*** we focussed on the reliability of the wound measurement technique. The aim of our study was to investigate the intra- and interobserver reliability of an instant full scale photographic technique combined with transparency tracing, avoiding the disadvantages of the separate components of this combination in measuring wound surface area. Duplicate photographic measurements of 30 wounds were obtained in 26 patients once a week over a period of two weeks, resulting in 120 photographs in total. Subsequently, duplicate tracing was assessed by two independent observers amounting to 480 observations. Patients were recruited from three long term care facilities. This study used the Intraclass Correlation Coefficient (ICC) as an indicator of chance-corrected agreement to estimate the reliability for the intra- and interobserver data. Additionally an Bland-Altman plot was constructed to measure the relationship between interobserver differences and wound surface area.
Analysis of the data revealed that all measurement comparisons were highly reliable; ICCs=0.99. No statistical differences between measured surface areas could be demonstrated. Linear regression showed a very small, albeit clinically unimportant, association (ß=0.0027; 95% CL 0 to 0.005) between interobserver disagreement and the size of the wound.
We conclude that he described method represents a simple, practical, and inexpensive technique to accurately monitor and evaluate healing of pressure ulcers over time and should be used in preference to separate transparency tracing or photographic techniques. Our results indicate that measurements obtained with this combined method are highly reliable ***within*** and ***between*** observers.

In view of the absence of randomized studies with sufficient large sample sizes, in ***Chapter 5***, we assessed the efficacy of LLLT in the treatment of stage III decubitus ulcers. We performed a prospective, observer blinded, multicenter, randomized clinical trial to assess the effect of LLLT as adjuvent to standard decubitus care. A total of 86 patients were enrolled into the study. Treatment was the prevailing consensus decubitus treatment (n=47); one group (n=39) had LLLT in addition, five times a week over a period of six weeks.
The primary outcome measure was the absolute (mm^2) and relative (%) wound size reduction at six weeks compared to baseline. Secondary outcome measures were the number of patients developing a stage IV ulcer during the study period, and the median change in Norton scores at six weeks compared to baseline.
Mann Whitney U tests showed that the differences between the two groups in terms of absolute improvement (p=0.50) and relative improvement (p=0.40) were not significant. Because the wound size areas were non-normally distributed, we additionally analyzed the data after logarithmic transformation of the wound size measurements. No significant difference in $\log_e$ improvement scores between both groups could be demonstrated

(unpaired *t*-test: *p*=0.64). During the treatment period 11% of the patients in the control group, and 8% of the patients in the LLLT group developed a stage IV decubitus ulcer (Fisher's exact test: *p*=0.72). The patients' Norton scores did not change during the treatment period.
In this trial, we found no evidence that justifies using Low Level Laser Therapy as an adjuvant to the consensus decubitus ulcer treatment.

In ***Chapter 6*** we went back to the basis of LLLT research in wound healing. Based on results of cell studies and animal experiments, clinical trials with Low Level Laser Therapy (LLLT) were performed, which finally did not demonstrate a benificial effect on outcome of wound healing. The aim of this study was to investigate whether the evidence from cell studies and animal experiments with respect to wound healing was unequivocally in favour of LLLT, which would imply that these models might be adequate to predict treatment response in patients, or that the data of cell studies and animal experiments were inconclusive, which would mean that the clinical trials were based on insufficient evidence.
We performed a systematic review of cell studies and animal experiment with LLLT on wound healing. Manuscripts were identified by searching MEDLINE, EMBASE, and SPIE (the International Society for Optical Engineering). We assessed whether studies showed a beneficial effect of active treatment or not. The effect size was expressed in standardized mean difference (SMD [the mean outcome measure of the treatment group minus the mean outcome measure of the control group, divided by the pooled standard deviation of these measurements]).
In depth-analyses were perfomed on [1] studies in which inflicted wounds on animals were irradiated and evaluated; [2] studies with primary outcome measures on dimensions with direct reference to wound healing (ranging from acceleration of wound closure to epithelialization, but excluding surrogate dimensions with regard to wound healing; in this case: tensile strenght); [3] animal studies with 'true controls'; [4] studies in which animals functioned as their 'own controls' and [5] studies with the highest methodological quality score.
The 36 included studies contained 49 outcome parameters of which 30 reported a positive effect of laser irradiation and 19 did not. Eleven studies presented exact data about the effect of active treatment and controls. The pooled effect size (SMD) over 22 outcome measures of these studies was -1.05 (95% CI: -1.67 to -0.43) in favour of LLLT. Methodological quality of the studies was poor. In depth-analysis of studies showed no significant pooled effect size in studies with highest methodological quality scores (0.06 [95% CI: -0.42 to 0.53]).
Summarizing the data of cell studies and animal experiments, reviewed in this manuscript, these studies failed to show unequivocal evidence to substantiate the decision for trials with LLLT in large number of patients. In fact, there were no differences between the results of these experiments and clinical studies. Remarkably, we found that (almost from the introduction on) cell and animal experiments and clinical studies that adress the biological effects of LLLT on wound healing, ran simultaneously, rather than in sequence. We conclude that this type of phototherapy should not be considered a valuable (adjuvant) treatment for this selected, generally therapy-refractory condition in human.

Chapter 7 contains the general discussion. Although the published clinical studies frequently lacked essential details (such as irradiation parameters) and showed poor methodological quality (e.g. small sample sizes and limited blinding), high expectations arose when some of these studies found a positive effect of LLLT on several aspects of wound healing. However, only four randomized clinical trials were reported. The overall result from a meta-analysis of these latter studies showed no significant beneficial effect on wound healing. Moreover, the randomized clinical trial, presented in this thesis, neither showed a significant difference between the LLLT-group and the control group.

Cell studies and animal experiments were reported until 1998 and 2000, respectively, and ran parallel to clinical studies. In our post-hoc analysis, a dose related effect (J/cm^2), theoretically described as the Arndt-Schulz principle, could not be confirmed emperically. Some additional remarks are made with regard to the methods used in animal experiments. Loose skin rodents were the type of animals in most studies. Their wound healing process differs substantially from human wound healing. However, a more appropriate animal type (pigs) failed to show convincing evidence with respect to a benificial effect of LLLT. Because of a possible systemic effect, future studies must be carried out with animals not being their own controls.

A healing-impaired animal model (diabetic, or aged animals) in the would be more appropriate than fresh inflicted lineair incisions in young species. In conclusion, the available data from cell studies and animal experiments were not addressed critically, which too early led to clinical studies. At present, LLLT is not included in the Dutch consensus decubitus texts. To our opinion, inclusion in the distinguishable ‘useless’ category is worth considering.

Samenvatting

Samenvatting

In het inleidend ***Hoofdstuk 1*** worden de vier stadia van decubitus beschreven, alsmede de pathofysiologische, klinische en patiënt-gerelateerde risicofactoren voor de ontwikkeling ervan. Daarnaast worden de achtergronden van laagvermogen-laserbehandeling [Low Level Laser Therapy (LLLT)] beschreven als mogelijke behandeling voor chronische wonden.

In ***Hoofdstuk 2*** wordt een systematisch literatuuroverzicht (systematic review) gepresenteerd over de effectiviteit van infrarood laagvermogen-laserbehandeling op de humane wondgenezing. Met behulp van geautomatiseerde literatuurbestanden (MEDLINE, EMBASE, CINAHL, SPIE en de Cochrane Database) zijn artikelen getraceerd. Van alle gevonden artikelen zijn de referenties nagetrokken en tevens zijn congresverslagen, literatuuroverzichten en handboeken geraadpleegd. Vervolgens werden de artikelen beoordeeld op methodologische kwaliteit en werd de effectgrootte (gestandaardiseerde verschillen in effect tussen de bestudeerde interventies) berekend. De uitgebreide zoekacties naar gerandomiseerde onderzoeken leverden vier effectstudies op over LLLT versus placebo of willekeurig andere interventie. Slechts één onderzoek maakte melding van een gunstig effect. Op een 100-punts schaal varieerde de methodologische kwaliteit van slecht (29) tot onvoldoende (47). Afzonderlijk hebben al deze studies een geringe statistische bewijskracht vanwege de beperkte grootte van de onderzoeksgroepen. Op drie studies was het mogelijk een meta-analyse uit te voeren.
De totale schatting van de effectgrootte toont aan dat laagvermogen-laserbehandeling geen statistisch significante bijdrage levert aan de wondgenezing (pooled RR=0,76; 95% CL 0,41 tot 1,40). We stellen daarom vast dat er geen wetenschappelijke argumenten zijn voor de toepassing van infrarode laserbestralingen (904 nm) bij patiënten met decubitus, ulcus cruris of andere chronische wonden.

Hoofdstuk 3 beschrijft een gerandomiseerde pilot-studie uitgevoerd in vier verpleeghuizen. Het onderzoeksdoel was vierledig: [a] ter evaluatie van de haalbaarheid van een 'multicenter study' in verpleeghuizen; [b] ter beoordeling van de toepasbaarheid van het meetinstrument; [c] ter beoordeling van de wondgrootte in beide onderzoeksarmen ten behoeve van adequate 'power analyse' voor toekomstig vervolgonderzoek; en [d] ter bepaling van het behandeleffect van een gallium aluminium (GaAl) 904 nm cluster laserbestraling (bestaande uit 12 laser dioden) met 1 J/cm^2 op weefselregeneratie van decubitus wonden in stadium III.
In totaal werden 20 patiënten voor deelname gerecruteerd. Daarvan werden 16 patiënten gerandomiseerd, vier patiënten voldeden niet aan de inclusiecriteria. De behandeling bestond uit de vigerende consensus decubitusbehandeling (*n*=8), terwijl een andere groep (*n*=8) de 904 nm laagvermogen-laserbestraling daaraan kreeg toegevoegd gedurende een periode van 6 weken met een behandelfrequentie van vijf keer per week. De primaire uitkomstmaat was de mediane wondgrootte op 6 weken na interventie.

Er werden geen statistisch significante verschillen gevonden ***tussen*** de twee groepen (Mann Whitney *U* test; p=0,47). In vergelijking met de baseline-registratie was de mediane afname van de wondgrootte 83% in de LLLT-groep en 95% in de controlegroep. Er was wel een significant verschil in afname van de wondgrootte ***binnen*** de onderzoeksarmen (Friedman Two-way Analysis; beide p<0,001).
We concluderen dat een multicenter studieopzet terdege haalbaar blijkt te zijn in de verpleeghuissituatie, terwijl de registratiemethode gemakkelijk uitvoerbaar en nauwkeurig bleek. Grootschalig klinisch onderzoek is noodzakelijk om de effectiviteit van LLLT te kunnen aantonen. Ter voorbereiding van een dergelijke studie berekenden we dat een groepsgrootte van tenminste 74 patiënten (37 personen per onderzoeksarm) noodzakelijk is om een verbetering van log 0,3 delta ten gunste van de experimentele groep te detecteren met een tweezijdig significantieniveau (alpha) van 0,05 en een power van 0,80.

In ***Hoofdstuk 4*** hebben we ons gericht op de betrouwbaarheid van de wondmeting. Het onderzoeksdoel van de studie was het bepalen van de intra- en interbeoordelaarsbetrouwbaarheid van een instant 1:1 fotografische techniek, gecombineerd met oppervlaktemeting via transparante (gerasterde) folie. Deze combinatie voorkomt de nadelen die aan elk van de twee afzonderlijke componenten verbonden zijn.
Gedurende een periode van twee weken werden wekelijks twee fotografische registraties van 30 wonden, afkomstig van 26 patiënten, verricht, resulterend in 120 foto's. Vervolgens vonden twee oppervlaktemetingen plaats door twee onafhankelijke beoordelaars, resulterend in een totaal van 480 metingen. De patiënten werden gerecruteerd uit drie verpleeginstellingen.
Voor de statistische analyse ter bepaling van de intra- en interbeoordelaarsbetrouwbaarheid werd gebruik gemaakt van de Intraclass Correlation Coefficient (ICC). Daarnaast stelden we een Bland-Altman grafiek samen ter bepaling van het verband tussen de interbeoordelaarsverschillen en de wondgrootte. Uit de data-analyse kwam naar voren dat alle vergelijkingen van de verrichte metingen betrouwbaar bleken; ICC's=0,99.
Er bleken geen statistisch significante verschillen tussen de oppervlaktemetingen te bestaan. Lineaire regressie-analyse toonde een geringe, maar klinisch onbelangrijke, relatie aan tussen de interbeoordelaarsverschillen en de grootte van het wondoppervlak (ß=0,0027; 95% CL 0 tot 0,005).
Concluderend blijkt de beschreven wondregistratie-techniek een eenvoudige, praktische en goedkope methode om het genezingsproces van decubituswonden vast te leggen en te evalueren. De methode verdient de voorkeur boven de afzonderlijke transparante folie- en fotografische registratie. De met deze gecombineerde techniek verkregen onderzoeksresultaten blijken, zowel ***binnen***, als ook ***tussen*** beoordelaars, betrouwbaar.

Gelet op het ontbreken van gerandomiseerde studies met een onderzoekspopulatie van voldoende omvang, beoordeelden we in ***Hoofdstuk 5*** het effect van laagvermogenlaserbestraling bij de behandeling van decubituswonden in stadium III. Daartoe verrichtten we

een prospectieve, geblindeerde en gerandomiseerde multicenter studie, waarbij LLLT werd toegevoegd aan de consensus decubitusbehandeling. In totaal participeerden 86 patiënten in het onderzoek, daarvan ontvingen 47 patiënten de vigerende standaard decubitusbehandeling, terwijl 39 patiënten de LLLT-behandeling daaraan kregen toegevoegd. De behandelfrequentie bedroeg vijf maal per week gedurende zes weken. De primaire uitkomstmaat betrof de absolute (mm^2) en relatieve (%) afname van het wondoppervlak in vergelijking met de baseline-registratie. De secundaire uitkomstmaten waren het aantal patiënten dat decubitus stadium IV ontwikkelde en de mediane verandering van de Norton scores in vergelijking met de baseline-meting. De uitslagen van de Mann Whitney *U* test toonden geen statistisch significante verschillen met betrekking tot de absolute (p=0,50) en relatieve (p=0,40) wondverbetering. Aangezien de grootte van de wondoppervlakten niet normaal verdeeld was, werd, na logaritmische transformatie, een additionele data-analyse van de wondoppervlaktemetingen uitgevoerd. Daarbij konden geen significante verschillen in $\log_e$ scores worden aangetoond in wondverbetering tussen beide groepen (ongepaarde *t*-test: p=0,64).
Tijdens de trial-periode ontwikkelde 11% van de patiënten in de controlegroep en 8% van de patiënten in de LLLT-groep decubitus stadium IV. De Norton scores bleven gedurende de behandelperiode onveranderd.
Wij hebben geen aanwijzingen kunnen vinden die het gebruik van laagvermogen-laserbestralingen, toegevoegd aan de consensus decubitusbehandeling, rechtvaardigen.

In ***Hoofdstuk 6*** zijn we teruggegaan naar de wetenschappelijke basis van laagvermogen-laser bestraling bij wondgenezing. Gebaseerd op de resultaten van celstudies en dierexperimenteel onderzoek, werden klinische trials met LLLT verricht die uiteindelijk geen positief effect op wondgenezing lieten zien. Het doel van het in dit hoofdstuk beschreven onderzoek was om na te gaan of het bewijs van celstudies en dierexperimenteel onderzoek met betrekking tot wondgenezing eenduidig in het voordeel van LLLT was, hetgeen zou betekenen dat deze modellen toereikend zijn om het klinisch effect bij patiënten te voorspellen. Dan wel, dat de celstudies en het dierexperimenteel onderzoek geen eenduidige resultaten lieten zien, hetgeen impliceert dat de klinische studies gebaseerd waren op onvoldoende wetenschappelijke bewijskracht.
We voerden een systematisch literatuuroverzicht (systematic review) uit van celstudies en dierexperimenteel onderzoek met laagvermogen-lasers bij wondgenezing. MEDLINE, EMBASE en SPIE (the International Society for Optical Engineering) werden geraadpleegd ter identificatie van artikelen. Vervolgens werd nagegaan of de studies al dan niet een positief effect op actieve LLLT-behandeling lieten zien. De effectgrootte werd uitgedrukt als gestandaardiseerd gemiddeld verschil (standardized mean difference [SMD]); de gemiddelde uitkomstmaat van de behandelde groep minus de gemiddelde uitkomstmaat van de controlegroep, gedeeld door de 'gepoolde' standaarddeviatie van die metingen. Daarnaast richtten meer gedetailleerde analyses zich op [1] studies, waarbij op dieren aangebrachte wonden werden bestraald en gemeten; [2] studies met directe wondgenezingsgerelateerde kenmerken als primaire

uitkomstmaat (variërend van snellere wondsluiting tot epithelisatie, maar afgeleide wondkenmerken - in casu de trekvastheid - werden uitgesloten); [3] dierexperimenteel onderzoek met een onafhankelijke controlegroep; [4] experimenteel onderzoek, waarbij dieren hun eigen controle vormden; en [5] de studies met de hoogste methodologische kwaliteit.
De 36 geïncludeerde studies bevatten 49 uitkomstmaten, waarvan er 30 een positief effect op laagvermogen-laserbestraling lieten zien en 19 niet. Elf studies presenteerden precieze gegevens over het effect van de actief behandelde groep en hun controlegroep. De 'gepoolde' effectgrootte (SMD) over 22 uitkomstmaten uit deze studies was -1,05 (95% CI: -1,67 tot -0,43) ten gunste van LLLT. De methodologische kwaliteit van de studies was slecht.
Gedetailleerde analyse resulteerde in een statistisch niet-significante 'gepoolde' effectgrootte onder de studies met de hoogste methodologische kwaliteit [0,06 (95% CI: -0,42 tot 0,53)].
Samenvattend stellen we vast dat de uitkomsten van de onderzochte celstudies en dierexperimenteel onderzoek geen overtuigend bewijs lieten zien die de stap naar klinische studies onder (relatief grote) groepen patiënten rechtvaardigde. In feite zijn er geen grote, betekenisvolle verschillen tussen de resultaten van deze experimenten en de resultaten van klinisch onderzoek. Het is verrassend dat (welhaast vanaf de introductie) de cel- en dierexperimenten met betrekking tot LLLT en wondgenezing parallel liepen met klinische studies, terwijl men zou verwachten dat het klinisch onderzoek in zekere mate voorafgegaan wordt door celstudies en dierexperimenteel onderzoek. We concluderen dat dit type lichttherapie niet beschouwd kan worden als een waardevolle aanvulling op de behandeling van deze, in de humane situatie veelal therapie-resistente, aandoening.

Hoofdstuk 7 bevat de algemene discussie. Hoewel in gepubliceerd klinisch onderzoek dikwijls essentiële informatie ontbrak (zoals de bij de bestraling gebruikte doseringsparameters) en het daarnaast gekenmerkt werd door een slechte methodologische kwaliteit, werden hoge verwachtingen gewekt toen enkele van die onderzoeken een positief effect van LLLT op wondgenezing rapporteerden. Er werden echter slechts vier gerandomiseerde klinische studies gevonden, waarvan het uiteindelijk via meta-analyse verkregen resultaat geen algemeen statistisch significant voordelig effect ten aanzien van wondgenezing liet zien. Daarnaast toonde de in dit proefschrift opgenomen gerandomiseerde klinische trial evenmin significante verschillen tussen de LLLT-groep en de controlegroep.
Celstudies en dierexperimenteel onderzoek werden beschreven tot respectievelijk 1998 en 2000 en liepen parallel met klinisch onderzoek. In onze post-hoc analyse kon een dosis-effect relatie (J/cm^2), theoretisch beschreven als het Arndt-Schulz principe, niet empirisch worden vastgesteld. Daarnaast werden enige aanvullende kanttekeningen geplaatst met betrekking tot de toegepaste methode in de dierexperimenten. In de meeste studies werden knaagdieren met een losse huid gebruikt. De wondgenezing bij dergelijke dieren verschilt echter substantieel van het humane wondgezingsproces. Met een geschikter diermodel (varkens) slaagde men er overigens evenmin in om een overtuigend bewijs van een gunstige invloed van LLLT op wondgenezing aan te tonen. Vanwege een mogelijk systemisch effect zou toekomstig

onderzoek moeten worden uitgevoerd met proefdieren die niet hun eigen controle vormen. Tevens zou het gebruik van een diermodel met vertraagde wondgenezing (diabetische, of oudere dieren) meer valide zijn dan 'vers' aangebrachte lineaire incisies bij jonge dieren. We concluderen dat de beschikbare gegevens weinig ondersteuning bieden aan de theoretische achtergrond. Daarnaast stellen we vast dat de beschikbare gegevens van celstudies en dierexperimenteel onderzoek niet voldoende kritisch werden beoordeeld, hetgeen te vroeg tot klinische studies heeft geleid. Momenteel is laagvermogen- laserbehandeling niet opgenomen in de Nederlandse consensus decubitus tekst. Naar onze overtuiging is opname in de categorie 'niet zinvol' het overwegen waard.

Dankwoord

Dankwoord

Dit proefschrift is te danken aan velen die hun kostbare tijd en waardevolle adviezen hebben gegeven, alsmede betekenisvolle bijdragen hebben geleverd aan de totstandkoming ervan. Het College van Bestuur, van de Hogeschool van Amsterdam, in het bijzonder Drs. W.M. Schoorl-Bouman, heeft het belang van wetenschappelijk onderzoek op het terrein van fysiotherapie onderkend. Zeer erkentelijk ben ik het College voor de ruimhartige condities waaronder ik mijzelf kon ontwikkelen. Ik voel mij dan ook zeer bevoorrecht dat ik op voorspraak van de Raad van Bestuur van het Academisch Medisch Centrum van de Universiteit van Amsterdam, met name Prof. Dr. N.A.M. Urbanus, op 27 november 1996 contact mocht leggen met de eerste van mijn twee aangezochte promotoren Prof. Dr. R.J. de Haan.

Hooggeleerde De Haan, beste Rob, ik heb grote bewondering voor je snelle en overstijgende wijze van denken. De vele claims die vanuit de laser-wereld werden gelegd, heb je van meet af aan kritisch aangehoord. Je hebt mij enthousiast ondersteund en je garant gesteld voor het welslagen van het onderzoek. Ik heb het zeer gewaardeerd dat je me de ruimte hebt gegeven voor eigen inbreng en verantwoordelijkheid ten aanzien van de opzet en uitvoering van de diverse onderzoeksonderdelen. Jij leerde mij de juiste woorden te vinden om al die wervelende gedachten in mijn hoofd op papier te krijgen. Je adviezen over vorm, stijl en eenvoud van wetenschappelijk werk mogen daarbij niet onvermeld blijven. Ik hoop oprecht dat wij elkaar niet uit het oog verliezen.

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* Vrij naar C. van Doesburg

Curriculum vitae

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Cees Lucas werd in november 1951 te Amsterdam geboren. Na het behalen van het HBS diploma aan de Christelijke Scholengemeenschap 'Pascal' te Amsterdam, vervulde hij zijn militaire dienstplicht bij de Geneeskundige Troepen. Daarna studeerde hij fysiotherapie aan de Academie voor Fysiotherapie 'Jan van Essen' te Amsterdam. Aansluitend op die studie volgde in 1978 een aanstelling als docent Fysische Therapie i.e.z. aan diezelfde academie, in combinatie met onderwijskundige en didactische scholing. Tijdens de fusie van de drie Amsterdamse Academies voor Fysiotherapie nam hij gedurende vier jaar de directietaken waar aan de expirerende Academie voor Fysiotherapie 'Jan van Essen', om daarna als onderzoeksmedewerker en docent aangesteld te worden aan de Faculteit Gezondheidszorg van de Hogeschool van Amsterdam. Thans is hij werkzaam als onderzoeksmedewerker aan de Afdeling Onderzoek en Innovatie Gezondheidszorg en als senior-docent aan het Instituut Fysiotherapie van de Hogeschool van Amsterdam. Hij doceert Methodology & Statistics, alsmede Scientific Research in de Engelstalige studierichtingen (American Stream Physical Therapy, Ghana Physiotherapy Education Program en European School of Physiotherapy). Tevens verzorgt hij de methodologische begeleiding van diverse onderzoeksprojecten op het gebied van fysiotherapie en revalidatiegeneeskunde. Voor het Ministerie van VWS beoordeelt hij de kwaliteit van fysiotherapeutische, ergotherapeutische en logopedische expertise van buitenlandse diplomahouders. Daarnaast werkt hij als fysiotherapeut in zijn eigen, bescheiden praktijk te Nieuw-Vennep. In mei 1997 leidde intensief overleg met het College van Bestuur en de toenmalige Faculteitsdirectie van de Hogeschool van Amsterdam tot de felbegeerde toezegging tot 'deeltijd studieverlof ten behoeve van het verrichten van promotie-onderzoek' resulterend in dit proefschrift.

Front cover photograph:

A 62 year old Japanese man takes care of his 91 year old mother. Over the entire body she suffers from decubitus ulcers, which he treats with a hair dryer. This takes three hours. Her hands are tied, preventing her from scratching the painful areas.

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