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Ultrasound Therapy for Wound Healing: A Review of Current Techniques and Mechanisms of Action

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Ultrasound (US) waves have shown promising therapeutic outcomes for different wounds. High penetration into the wound bed, highly steering and focusable and not approved harmful effects are the main advantages of US treatment for wounds. In addition to antimicrobial effects, triggering wound-healing physiological mechanisms are among the mechanisms of action of US in wound healing. Despite of rigorous evidence on the therapeutic efficiency of US in different and particularly chronic wounds, no definite dose-response existed on the clinical trials applications of this technique. However, there is a consensus on the spatial average temporal average dosage in the range of 0.5 W/cm2 to 3 W/cm2 with significant therapeutic outcomes and minimum adverse effects. Further in vitro and clinical trials are needed to shed light on the exact mechanisms of action and dose-response of US techniques for different wounds.

Key words: Ultrasound, Wound healing, Low Frequency Ultrasound, Mechanism of Action.

As well as conventional medications, different more-recent techniques have been developed for the treatment of wounds such as pressure relieving beds, cushions, medicinal plants. They are generally used as measures for prevention and treatment of pressure wounds. High worldwide prevalence of wounds, high costs of traditional methods and elimination of reimbursement for various wounds like burns, venous leg ulcers or infections have boosted the rapid raising of alternative wound healing methods. During the last decade several methods for chronic and acute wounds treatment including laser, electricity magnetic, light and electromagnetic that are being used for healing wounds and sores[1-8].

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Ultrasound (US) techniques are among the most recent methods for treatment of wound with promising outcomes. US based techniques have major advantages over conventional and other alternative techniques. US waves can penetrate into the beyond of the wound bed and reach more deep-seated tissues compared with other methods. Furthermore, the US waves can be highly oriented and focused compared with other techniques. The US waves are usually delivered to tissues through a saline mist.

Wounds are classified into two categories including acute and chronic. Majority of acute wounds can be healed by direct union while chronic wounds remain for an extended time. If a wound does not follow the normal model of healing which extends almost up to six weeks, it is considered a chronic wound[9].

Studies on the interactions between high frequency sound waves and living organs and tissues dated back seven decades. Various studies

have shown that US energy has therapeutic potentials[10]. Since the discoveries of potential therapeutic effects of US energy, various US technologies have been used for treatment of several disorders including skin wounds, malignant tumors, bone fractures[11, 12]. Advantages of US treatments have made them one of the most promising treatment options for the management of soft tissue injuries[13]. Many experimental studies have shown various physiological efficacies of US on living tissues[14-17] and also vigorous evidence indicating the beneficial effects of these mechanical waves in the treatment of soft tissue disorders[18-20].

In clinical experiments, US waves have theragnostic values in different diseases. In wound healing applications, both high (1-4 MHz) and low (20-120 KHz) frequencies of US are used.

Therapeutic US is a physical method delivering non-ionizing radiation in the form of mechanical sound waves into the tissues to produce heat the tissue. The therapeutic effects of US depend on dose (W/cm^2 time) and dosage (frequency of application, series)[21]. It is usually exerted at two fixed frequencies of 1.0 MHz and 3.0 MHz and is the most generally used deep-heating modality, able to attain depths of 5 cm and more below the surface of the body. The US, similar to short-wave diathermy, can be exerted in pulsed or continuous waves to apply therapeutic thermal and non-thermal efficacies[21].

Choice of the parameters of US techniques depends on the desired effect and the density and location of the tissue under treatment. These parameters are evaluated by physicists and therapists through conducting some experiments. Some applications of high frequency US treatment include treatment of tendon injuries and relief of the short-term pain[22-24]. Furthermore, US has been demonstrated to accelerate healing of some acute bone fractures, venous and pressure ulcers, and surgical incisions[22, 25, 26]. However, US treatment may cause burns or damage the endothelial under inappropriate parameters[27, 28]. In line with research progressions, different commercial modalities based on low frequency US were offered to the market. Usage of high frequency US in clinical medicine is restricted due to the risk of tissue heating. As a result, considerable research attempts have exploited alternative US parameters. Therefore, applying low-frequency US with less tissue heating, thereby operating as a "slow release" technique, may become the standard model of care in treating slow-to-heal wounds, skin ulcers and nonunion fractures.

This paper aims to review the current US techniques for wound healing and highlight their main mechanism of actions in wound healing processes. Furthermore, the efficacy of US treatment on various types of wounds is compared.

Biological Effects of US

High power, high frequency US is described as US of 0.5-10 MHz and with intensity up to 1500W/cm^2 whereas low power, low frequency US is determined as an US of 20-120 kHz and 0.05-1.0 W/cm^2. Low frequency/low intensity US is mainly reflected in the wound surface or skin. Only small fractions of the energy released by the probe are delivered to the deep-seated tissue layers and the main effect is mechanical effect, which is in contrast to high frequency US with combined mechanical and thermal effects.

Previous in vitro and in vivo studies on the therapeutic feasibility of low frequency US have indicated various clinical effects which are dependent on the exposure levels. High intensities can cause cell death, while at low levels reversible and useful effects are occurred.

The ''low power'' US techniques are used in physiotherapy, fracture repair, sonoporation, sonophoresis and gene treatment. Treatment efficacy through the intensity spectrum is acquired by both thermal and non-thermal interaction mechanisms. At low intensities, acoustic streaming is considerable, whereas at higher levels, thermal and acoustic cavitations are predominant effects. Although useful therapeutic effects are clinically demonstrated, the mechanisms of action of US are not fully understood.

In the physiotherapy applications, US is mainly utilized in the soft tissue hurts therapy, to increase the rate of wound healing, eliminate edema, soften scar tissue, bone injuries and circulatory disorders.

US techniques were originally considered as superseded diathermy therapy, competing with microwave, and radiofrequency and hot packs techniques to generate middle heating. As the basic understanding of all the therapeutic mechanisms

of US improves, treatment regimes are being altered in an attempt to make use of any beneficial non-thermal mechanisms that may exist (by use of lower intensities and of pulsed beams). There is a lack of scientifically designed controlled clinical experiments, and so the US therapy regime used is usually characterized by trial and error, and sometimes to each department's particular ''recipe''[29].

To develop and optimize efficient US treatments for wound it is necessary to know the exact mechanisms of action of US waves on the target tissue. Different systematic reviews of therapeutic US have shown no a dose-response relationship[30-31]. However, spatial average temporal average dosage with the range of 0.5 W/cm^2 to 3 W/cm^2 has been reported to minimize adverse effects[31]. Recently published randomized controlled trials which have reported significant benefits of therapeutic ultrasound over placebo ultrasound have used dosages of 1 W/cm^2 to 1.5 W/cm^2 [32-34].

There are rigorous evidence in the literature demonstrate that US high intensities can harm bone or delay healing[35, 36] and low intensities can increase rate of repair and decrease the time of curing[37, 38].

Low intensity pulsed US methods have therapeutic effects on different disorders like bone fracture healing, osteoporosis and pain relieving[39, 40, 41-44].

There is clinical evidence of the efficiency of very low intensity US on bone and soft tissue healing. At low intensities, thermal effects are not likely the responsible mechanism of action. US can enhance the penetration of pharmacologically-active drugs through the skin. This process where the infiltration of a drug is externally enhanced is known sonophoresis or phonophoresis[45, 46]. Although the exact mechanisms of sonophoresis induction is not determined, it is proposed that acoustic cavitation or streaming temporally makes the stratum corneum permeable increases perfusion[45-47]. Low US frequencies show more therapeutic efficiency in wound healing compared with high frequencies.

Sonoporation is a phenomenon where US transiently changes the cellular membrane structure and reversible pores are formed across the membrane so that the high molecular weight molecules can enter the cell. Several studies have demonstrated the synergistic efficacies of US and various drugs[46, 48].

However, an important issue should be carefully considered in interpreting the findings of in vitro studies: Acoustic cavitation and streaming are predominant phenomena in aqueous in vitro environment which is different with in vivo US exposure. Therefore, the mechanisms of action of acoustic cavitation and streaming are different in two mediums.

It has been suggested that streaming can facilitate the drug penetration into the clot, or that the US mechanical action can affect the fibrin mesh and results in better access for the drug.

Similarly, low frequencies US have the benefits of increased penetration into the skull that may be useful in stroke applications. Also, the US with the range of frequency between 26 kHz– 5 MHz has been studied[49]. However, at high intensities, US can enhance the deposition of platelet and fibrin. Investigating different intensities of US in the range of 1.1–3.2 W/cm^2 demonstrated that at 0.5–1 W/cm^2 these US produced clot lysis, while at 4 W/cm^2 there was lesser lysis of clot than in the attendance of fibrinolytic agents alone[50].

Leg ulcers are a major problem for patients and health service sources. Most wounds are correlated with venous diseases, but other causes or contributing factors contain immovability, obesity, trauma, arterial diseases, vasculitis, diabetes, and neoplasia.

During the last two decades, care management of patients suffering leg ulcers has improved.

Although US does not possess a direct anti-inflammatory effect, it seems that exposure to US during the initial 'inflammatory' phase of tissue repair can accelerate this phase.

The latter phase of healing is the 'proliferative' stage. In this stage, cells migrate to the injury site and begin to divide, granulation tissue is shaped, and fibroblasts start to create collagen. It has been demonstrated that US increases synthesis of collagen by fibroblasts and epithelium repairing[51-53].

The last phase of tissue remedy is 'remodeling'. In addition, there are some proofs that scar tissue cured with US may be more powerful

and elastic than 'normal' scar tissue.

Recently, data from clinical trials, case reports and observational results have showed that US can increase the rate of various ulcers healing through different mediators[54-56] . In addition, in a few cases in order to cure burn wounds, using low frequency US was also examined[57, 58]. Furthermore, since US is identified as a generator for diffuse of nitric oxide, it utilizes an auxiliary instrument for vasodilatation and palliation of pain in the treated wound[59, 60].

Characteristics of Therapeutic US

US includes inaudible high frequency mechanical vibrations produced when a generator produces electrical energy which is transformed to acoustic energy by mechanical deformation of a piezoelectric crystal placed in the transducer. The waves generated are transformed by diffusion and vibration of molecules, with a progressive loss of the intensity of the energy during passage through the tissue (attenuation), because of absorption, scattering or dispersion of the wave[61]. The main parameter for assessing the therapeutic efficacy of US techniques is the power expressed in Watts. The amount of energy attained by a particular site is dependent on the US characteristics (frequency, intensity, amplitude, focus, and beam uniformity) and the type and physical characteristics of tissues through which the US beam travels.

The frequency range of therapeutic US is 0.75–3MHz and most machines are set at the frequency of 1 or 3 MHz. Low frequency US waves have more penetration depth, but are less focused. One- MHz US is adsorbed primarily by tissues located in depth of 3–5 cm[62] that makes it ideal choice for deeper injuries and in patients with greater subcutaneous fat. The frequency of 3 MHz is applied for more superficial lesions at depths of l–2 cm [62, 63].

Tissues can be determined by their acoustic impedance, the product of their density and the speed at which US will transfer through it. Tissues with high-water content such as fat, have low absorption of US and thus high penetration of US waves, while tissues which are rich in protein like skeletal muscle have high US adsorption[64]. The larger acoustic impedance difference between two tissues, the less portion of the US wave will transmit through the interface[65]. When reflected US meets further transmitted waves, a standing wave may be generated, which has potential side effects on tissue[63]. Such adverse effects can be minimized by ensuring that the machine renders a uniform wave, using pulsed waves, and moving the transducer during the treatment process[64].

The greater diameter of the radiating area of the transducer face, the more focused US beam is generated. Energy is unevenly distributed within the US beam and the greatest non-uniformity occurs near the transducer surface. The beam intensity variation is determined by the beam non-uniformity ratio (BNR), the ratio of the maximal intensity of the transducer to the mean intensity across its surface[66].

Coupling media, in the form of water, oils and majority of gels, prevent reflection of the waves away at the soft tissue/air interface by removing air from between the patient and transducer. Each medium has its own impedance. Each coupling medium must have the same acoustic impedance to that of the transducer, should uptake few of the US, remain free of air bubbles and permit easy motion of the transducer over the surface of skin[67].

Dosage of US can also be changed by alteration of wave amplitude and intensity. In addition, therapeutic US can be continuous or pulsed. The continuous US exerts more heating effects. Pulsed US has on/off cycles, each component of which can varies to change the dose. At low intensity both forms produce non-thermal effects.

Physiological Effects of US

US energy produces a mechanical pressure wave through soft tissue. This pressure wave initiates two main processes: First, generation of microscopic bubbles in living tissues and distortion of the cell membrane, influencing ion fluxes and intracellular activity. Three main mechanisms of cell membrane distortion through US are acoustic streaming, bubble formation and microstreaming.

US can produce thermal and non-thermal physical effects in tissues. Non-thermal effects can be achieved with or without thermal effects. Thermal effects of US on tissue may enhance the blood flow, decrease muscle spasm, increase extensibility of collagen fibers and a pro inflammatory response. Thermal effects happen when the tissue temperature increases to 40–45 °C

for at least 5 min[68]. Extreme thermal effects, which are achieved in high US intensities, may hurt the tissue[64].

Previous in vivo and in vitro studies have shown that non-thermal effects of US, such as cavitation and acoustic microstreaming, are more significant in the treatment of soft tissue lesions than thermal effects[69]. Cavitation is the formation, oscillation, and collapse of bubbles under an US radiation force. In interstitial (tissue) fluids, ultrasonically induced pressure variations cause gas-filled bubbles expand and compress resulting in the enhancement of the flow in the surrounding fluid[70]. When bubbles expand and contract, without growing to critical size, stable cavitation is formed. Unstable cavitation does not occur in therapeutic range (pulsed 20% at 0.1 to 3 W/cm^2) in normal tissues except in air-filled cavities such as lungs and intestines. Stable cavitation is useful to damaged tissue, while unstable cavitation can damage tissue[71]. The stable cavitation can be suppressed with very short pulses. At least, 1000 cycles at 1 MHz are needed to instate stable cavitation[7]). Acoustic microstreaming, the unidirectional motion of fluids across membranes of cell, happens as a result of alteration of the mechanical pressure within the US field. Microstreaming may change the structure of cell membrane, function and permeability[65], which has been offered to stimulate tissue repair[69]. Some studies have demonstrated the effects of cavitation and microstreaming in vitro experiment such as stimulation of fibroblast repair and collagen synthesis[14-16, 72], regeneration of tissue[15] and bone healing[38].

Various mechanisms of action of US in modulating inflamed tissues including increasing the fibrinolysis rate[17, 73], stimulating macrophage derived fibroblast mitogenic factors[74], escalating fibroblast recruitment[11], accelerating angiogenesis[75], increasing matrix synthesis[72], synthesizing more dense collagen fibrils[76] and enhancing tissue tensile strength[16, 77, 78]. These interactions can interpret the usefulness of US in promoting and accelerating recovery of wound tissue. Although these results are related to wound healing, their relevance to tendinopathies, which represent a significant rate of soft tissue hurts, is unclear. Tendinopathies cover a wide range of histopathological characteristics from inflammatory lesions of the tenosynovium to degenerative tendinosis[71]. The degenerative procedure is poorly realized, but is considered to represent an internal tendon cells failure to repair and remodel the extracellular matrix after damage [79, 80]. Extensive studies of normal and degenerate human tendons have demonstrated striking alteration in composition of matrix[79-82], variation of collagen fiber type distribution, with a relative enhancement in type III collagen over type I collagen, and, in some tendon lesions, proliferation of fibrovascular and the focal expression of type II collagen, representative of fibrocartilaginous alteration. After damage, in order to remove damaged matrix and to remodel scar tissue, it is necessary to enhance matrix turnover. The efficacies of US on these procedures, that are themselves poorly realized, as yet are not identified.

Alternatively, US may be applied for its thermal effects to solace pain and muscle spasm to enhance the extensibility of tissue, that may be used in combination with stretching practice to gain optimal tissue length[83]. Lengthening with thermal doses of US has been shown in the ligament of normal knees[84] and in scar tissue[85]. When the tissue has been heated to an appropriate level which is between 43- 45°C [71], the chance to stretch the tissues lasts for up to 10-min prior the tissue cools[86].

Studies on the US applications specifically in tendon curing are limited and most of them are animal studies with inconsistent findings. Increases in strength of tensile, energy absorption, mobility, improved collagen fibril alignment, decrease in inflammatory permeate and scar tissue in tendons has been shown in some trials[87, 88] but not others[89, 90]. These studies altered considerably with respect to the regimes applied. Studies also show US treatment increases vasodilatation, stimulates vascular endothelial growth factor and angiogenesis, promotes early release of growth factors, and provides greater amounts of high-quality collagen. The overall result of these cellular effects is accelerated healing.

Ultrasound and Wound Healing

Based on in vitro and in vivo studies, the mechanisms of action of US treatment on wound healing can be specified for two distinct phases of wound recovery process:

Inflammatory Phase

The non-thermal effects of US induce mast cells degranulation. Mast cells release histamine and other chemical mediators. These mediators play an important role in absorbing neutrophils and monocytes to the injured site. These processes along with other events appear to increase the rate of acute inflammatory phase and promote wound healing[11, 74, 75].

Proliferative Phase

US techniques have been reported to affect fibroblasts which secrete collagen. Continuous US at higher intensities can heat deeper tissue more effective prior to stretch. As with other procedures of therapeutic heat, the US usage in this capacity is thought to enhance extensibility of collagen, circulation, pain threshold, enzymatic activity, permeability of cell membrane, acceleration of nerve conduction[91].

Physicians report that covering the wound area with a hydrogel film and applying US during the inflammatory and proliferative stages stimulate the cells involved in wound curing, warm the tissue, and increase healing by improving circulation[92].

It has been demonstrated experimentally in rat fibulae that when US exposures are conducted during the inflammatory and early proliferative phases of bone remedy following fracture, the rate of healing can be increased, with direct ossification being perceived. If remedial is delayed until the late proliferative phase, it is cartilage growth that is stimulated. It has been demonstrated that 1.5 MHz US could be more effective than 3 MHz (ISATP μ0.5 W/cm^2, pulsed 2 ms: 8 ms for 5 min)[93].

Effectiveness of Ultrasound Wound Treatment

Wound Healing- Angiogenesis

In vitro studies on the tunneling or debilitation wounds and surface model demonstrated that US can eliminate multi-drug resistant bacterial organisms. Organisms like Vancomycin-resistant Enterococcus and resistant Pseudomonas aeruginosa in vivo were cultured and cured with different US outputs and exposure times[94]. In vitro findings demonstrated that US treatment can enhance in vitro cell proliferation, collagen/ NCP production, formation of bone, and angiogenesis[95]. Other similar studies have assessed the various US machines on wound treatment and proved the angiogenesis effects of US[83].

Chronic Wounds

Management of chronic wounds related pain is a long standing clinical challenge in patient care and there is no definitive solution for treatment of chronic wounds related pain[58]. Different studies revealed that low frequency US is a useful device for chronic wounds management, not only for curing but also for pain relieving, pigmentation and odor decrease[96]. However, findings of similar studies on chronic wounds are controversial. A systematic review of the efficacy of different US modalities on wound care management deduced inadequate evidence for clinical efficacy of therapeutic US in chronic wounds[28].

According to the primary clinical evidence, patients with painful chronic lower-extremity wounds reported a wound pain reduction following US therapy. A low-frequency, non-contact US device for wound treatment, was approved for marketing by the United States Food and Drug Administration[58].

Purulent Wounds

Low frequency US techniques have been used in combination with standard wound care drugs or alone for treatment of purulent wounds. The findings of these studies showed the therapeutic effectiveness of US technique as an adjunctive or alternative treatment for purulent wound[97-99].

A case series study assessed the effectiveness of the combination of low frequency US together with gentamicin solution in 17 patients. This study revealed a reduction in the purulent septic complications from 35.7% to 5.9%[99].

A cross sectional experiment on 112 patients with diabetes mellitus and purulent surgical wound who were cured with low frequency US and laser radiation showed that US treatment had privileges in the first and second phases of wound curing procedure[97]. Another study revealed that an US surgical device "SUGA -21f.02" applied in 76 patients and an intensification of diffusion of the medical preparation into the tissues was demonstrated among the deep layers of the wound channel[98]. In a study which assessed the impact of US at two power densities of the range of 0.5W/ cm^2 and 1 W/cm^2 in the crural ulceration curing found that there was no significant difference in terms of granulation

development rate and debridement of the wound.

Trophic Ulcers

Gostishchev *et al*. (1984) investigated the effects of low frequency US on the trophic ulcers. They assessed clinical, morphological and medium pH measurement and showed granulation tissue growth which allows fulfilling autodermatoplasty[91]. Other studies have tested the efficacy of low frequency US applied in combination with antibiotic. In this area, Radiske *et al*. (2000) demonstrated that continuous US and systemic gentamicin administration significantly decreased the viable bacteria concentration in the simulated implant putridity[100], whereas some scientists (Qian *et al*. 1997; Pitt *et al*. 1994) have reported that application of US in the bacterial cultures of E. coli and P. aeruginosa increased the efficacy of gentamicin[101, 102].

Pressure Ulcer

In a systematic review conducted by Flemming *et al*. (2004) showed no vigorous evidence on the therapeutic efficacy of US in the pressure sores. They attributed the inconsistency to the variations and limitations in the methodology and the small sample size of the reviewed studies [103].

Reit *et al*. (1995) conducted randomized controlled trials of US treatments in patients with pressure ulcers. They observed no significant differences between treatment groups (David *et al*. 1996, Riet *et al*. 1995)[104].

In a review of pressure ulcers therapy, Reddy *et al*. (2008) performed a randomized controlled clinical trial and found no significant proof for the US efficacy in the treatment of pressure ulcers. The review of randomized controlled clinical trials found no clear evidence for the effectiveness of US in healing of pressure ulcers[105]. Akbari Sari *et al*. (2006) reviewed the effectiveness of US therapy on pressure ulcers. They showed no reliable proof of advantage of treatment by US in the healing of pressure ulcers. However, the feasibility of useful or harmful effect cannot be ruled out because of the small number of participants or other methodological limitations. Therefore, further studies are needed to reach a conclusive answer[103].

Extremity Lower Wound

Extremity lower wounds are among the most common types of wound worldwide[106]. US techniques have shown therapeutic efficacies for this type of wounds. Callum *et al*. (1987) applied weekly pulsed US therapy and compared this technique with standard wound care for chronic leg wounds. They used a 12-week treatment period and reported that the ratio of wound healed was 20% greater in the US group[107]. In a randomized controlled trial conducted by Lundeberg *et al*. (1989) to investigate the efficacy of pulsed US in conjunction with a standard technique of curing chronic leg ulcers therapy on 44 patients[108]. All patients received standard treatment (paste impregnated bandage and a self-adhesive elastic bandage) plus placebo US or pulsed US three times a week for 4 weeks. Then it was applied two times in a week for 4 weeks and once-weekly for the following 4 weeks. The rates of cured wound were tested after 4, 8 and 12 weeks[108]. They showed no significant differences in the percentage of cured ulcers in the pulsed US treatment as compared with the placebo group [108].

In another randomized controlled study, Eriksson *et al*. (1991) investigated the efficacy of US against the standard model of chronic leg ulcers healing. All patients received standard treatment plus placebo US with the intensity of 1.0 W/cm^2 at 1 MHz, for 10 mins twice a week for 8 weeks. The percentage of cured wound area and the number of healed wounds were compared after 2, 4, 6 and 8 weeks. The authors observed no significant differences in the percentage of cured ulcers in the US treatment group as compared with the placebo group[109]. Peschen *et al*. (1997) examined the effect of low-frequency (30 kHz) low-dose US on the chronic venous leg ulcers treatment in combination with conventional outpatients' therapy. Patients were randomly divided into conventional treatment with topical application of hydrocolloid dressings and compression therapy (conventional treatment plus US treatment). The US therapy included 10-min of foot-bathing with continuous US wave 100 mW/cm^2 thrice-weekly for three months. The ulcer area was measured by planimetry, using a millimeter grid before therapy and after 2, 4, 6, 8, 10 and 12 weeks of treatment. The group evaluated the radius of ulcer and the daily decrease of ulcer radius. After each therapy, adverse effects were recorded. After the period of treatment the mean decrease of ulcerated area in control group patients was 16.5% while this factor in the US group was 55.4 %. The daily decrease of ulcer size in the US-treated group

was 0.08 mm whereas this ratio in the placebo patients was 0.03 mm. Both US and placebo groups just recorded minor adverse effects. The authors concluded that the low-frequency and low-dose US technique is a beneficial therapeutic method in chronic venous leg ulcers[110].

Johannsen *et al*. (1998) carried out a meta-analysis study in order to explain the effect of chronic leg ulcer treatment by applying US. The result of their study demonstrated that when US was delivered in low doses around the ulcer edge, it has the best[111]. In a randomized, double-blinded, controlled, multi-center study, Ennis *et al*. (2005) performed a randomized double blinded, controlled, multi center experiment and they tested the efficacy of MIST US treatment for the recalcitrant diabetic foot wounds therapy. This study was conducted on 55 patients and they received standard care, which consisted of products that prepare a moist environment, without using diabetic shoes and socks, debridement, as well as wound assessment. The "treatment" was done using US wave of 40 kHz frequency achieved by a saline mist or a "sham device" that delivered a saline mist without applying US. This procedure was performed during 3 months and at the end of this period, the ratio of wounds healing which was performed by the US therapy device group was significantly higher than that in the control group. In this experiment no difference was observed in the number and type of side effects between the two groups. The authors explained that as compared with control, MIST US treatment accelerated the healing rate of diabetic foot ulcers. The results of the study demonstrated the need for further research, including assessing the impact of quantitative biopsy results at enrollment, debridement depth and impact on healing, as well as the potential anti-microbial action of MIST US therapy[112].

Ennis *et al*. (2006) assessed the effectiveness of MIST US on the occurrence of wound closure for chronic non-healing lower extremity wounds of different etiologies. Their study showed the appropriate and optimal therapy duration with this device quantified end points that correlated with a maximal clinical response and identified potential synergistic therapies that could be used in conjunction with this therapy. In addition, they investigated the effect of MIST US treatment on the micro circulatory flow patterns within the wound bed. Control data were obtained from a previously published, prospectively gathered database. This experiment lasted for 8 months. A total of 29 lower extremity wounds which were observed in the 23 patients who met criteria for inclusion were treated with MIST US treatment. Standard treatment period was prepared for 2 weeks for all wounds observed for the experiment. A breakage to obtain an area of reduction more than 15 % qualified the patient for registering the trial and the addition of MIST US therapy to the current cure regimen. The criteria for inclusion were the decrease of wound healing, area and volume, and laser Doppler-derived mean voltage. Totally, 69 % of the wound was healed by applying the desired therapeutic model. When MIST US was applied alone, the average time which was required for healing was 7 weeks, whereas the mean time to heal control groups was 10 weeks. The outcomes of this experiment demonstrated that using MIST US treatment alone or in combination with moist wound care could attain healing in 69% of chronic wounds. Non-contact US was evident within 4 weeks of therapy. The authors demonstrated that a well-designed clinical experiment based on health economics is required to evaluate this method[113].

Kavros and Schenck (2007) performed a non-randomized, baseline-controlled clinical case series study in order to describe the efficacy of non-contact low-frequency US treatment for chronic, rebellious lower-leg and foot ulcer. They first treated patients were initially treated with the Mayo Clinic standard method of care, and then they combined low-frequency US therapy with the former approach. They surveyed the medical records of 51 patients with one or more conditions cited below: diabetes mellitus, neuropathy, limb ischemia, chronic renal insufficiency, venous illness, and inflammatory connective tissue disease. All patients had leg and foot ulcer with 65% of patients having mellitus and 20 % of them having a history of amputation. 63 % of all the wounds had a multi-factorial etiology, and 65 % had correlated transcutaneous oximetry levels less than 30 mmHg. The mean time of wounds therapy during the baseline standard of care control period was almost 9.8 weeks while the non-contact US therapy of low frequency time was approximately 5.5 +/- 2.8 weeks. The results showed that utilizing

non-contact low-frequency US could improve the curing and closure in recalcitrant leg and foot ulcer[114].

In a randomized controlled trial study, Kavros *et al*. (2007) applied the MIST-US to treat non-healing leg and foot ulcers associated with ischemia of chronic critical limb. The treatment protocol consisted daily five-minute treatment for three times a week continued for three months or until wounds achieved full recovery. The main outcome measure was the percentage of patients with greater than 50 % falling in size of wound from the index of measurement after 3 months of treatment. The correlation between transcutaneous oximetry pressure in response to low-frequency of the supine and dependent position was assessed as a factor in evaluating the ability to heal the ischemic foot and leg ulcer. A significantly higher percentage of patients cured with the standard of care plus MIST US therapy obtained greater than 50% wound healing at 3 months than those which were just treated with the standard care. Therefore, failing to reach the minimal wound healing requirement happened in 37% of subjects in the treatment group and 71% of patients in the control group. The predictive value of baseline transcutaneous oxygen pressure may profit the clinician when evaluating the ability to ischemic wounds healing. The authors demonstrated that when they applied MIST US addition to the standard wound care model, the velocity of cutaneous foot and leg ulcer healing in patients with chronic limb ischemia cured greatly. It should be considered that though the study discussed the significance of baseline transcutaneous oxygen pressure on therapeutic wound, patients with high (21 to 40 mmHg) and low (1 to 20 mmHg) transcutaneous oxygen pressure levels are not equally distributed equally between the groups[54].

The American Society of Plastic Surgeons evaluated the efficacy and feasibility of US treatment for leg and foot ulceration[115]. Their assessments which were based on the clinical experiment guideline on chronic wounds of the leg and foot ulcer (2007) did not note the application of US as a choice of treatment[115].

Kavros *et al* (2008) performed a retrospective analysis, evaluating the clinical efficacy of non-contact, low-frequency MIST US in the chronic leg and foot ulcer therapy. They authors observed that a significantly larger percentage of wounds were healed by MIST US therapy plus standard wound care compared with the standard care alone. In addition, application of the MIST US therapy accelerated the rate of wound healing compared with the standard model. The authors concluded that when MIST US therapy was added to standard wound healing model, the rate of curing and closure of chronic wounds significantly improved. They concluded that the combine MIST US and standard wound care model accelerates the healing of chronic wounds in the leg and foot ulcer[116].

Cullum *et al*. (2010) conducted a Cochrane review on the efficacy of US on the rate of venous leg ulcer healing. They concluded that venous leg ulcers trial assessment with US is small, poor-quality and heterogeneous. There is no trustworthy proof that US improves venous ulcers healing. There is a number of weak evidence which showed enhanced curing with US; however, to reach a more conclusive answer, further confirmation in larger, high-quality RCTs is required[117].

Game *et al*. (2012) stated that the result of diabetic foot ulcers administration is poor, and there is continuing doubt concerning optimal methods to management. For this reason, in 2006 the International Working Group of the Diabetic Foot (IWGDF) group carried out a systematic review of the proof to inform protocols for current care and to highlight areas which must be considered for further research. This group updated the review by considering papers, published between December 2006 and June 2010, on the interventions to improve the chronic ulcers healing. Two research groups independently evaluated methodological quality of the selected studies using Scottish Intercollegiate Guidelines Network criteria. The selected studies were divided into ten categories: (1) sharp debridement and preparation of wound bed with hydrotherapy; (2) preparation of wound bed by using antiseptics, applications and dressing crops; (3) the chronic wound elimination; (4) HBOT; (5) compaction or negative pressure treatment; (6) designing products to correct aspects of biochemistry of wound and cell biology which associated with impaired wound curing; (7) cells application, containing stem cells and platelets;

(8) bioengineered skin and skin bounds; (9) magnetic, electrical, electromagnetic, lasers, shockwaves and US; and (10) other systemic treatments which were not found in the mentioned categories. Major challenges which prevented pooled analysis of outcomes are heterogeneity of experiments. With the exception of HBOT and, feasibly, negative pressure wound therapy, there is little evidence to justify the usage of newer treatments. This conclusion is consisted with a recent Cochrane review and the systematic review by the NICE Guidelines Committee in the United Kingdom. Analysis of evidence showed considerable difficulties in this field particularly as controlled studies are few and the majority suffer poor methodological quality[118].

Gottrup and Apelqvist (2012) noted that foot ulcer administration in patients suffering diabetes still remains a main remedial challenge all over the world. The authors carried out a review of available evidence and new methods in the diabetic foot ulcer therapy. The golden standard for optimal evidence in the Cochrane system is level I - RCTs, and meta-analyses of several RCTs. The results on several kinds of wound debridement, use of anti-microbial, use of dressings in wounds; topical negative pressure; hyperbaric oxygen treatment; electrical, electromagnetic, laser, shockwave, and US therapies, growth and cell biology factors, cell products and tissue engineering, bioengineered skin and skin grafts, and adjuvant therapies were evaluated. Review of the current literature demonstrated that there is restricted proof on the highest level to legitimize a variation in usual clinical experiment. There is a lack of high-quality proof, due to the studies mostly based on insufficient sample size, short follow-up, non-random allocation to treatment arms, non-blinded outcomes evaluation, poor description of control, and simultaneous intervention. The heterogeneity of the population of ulcer and people, with multiple factors help both ulcer beginning and failure to heal. Another main cause for the lack of evidence is the general utility of the measure of "complete healing". Therefore, it is necessary to enhance the quality and methodology of clinical trials[119].

Furthermore, there are some case studies as well as case-series studies on the application of non-contact, low-frequency US in the management of several kinds of chronic wounds like burns, digital ulcers, infected surgical wounds, and sacral pressure ulcers[57, 120-125].

CONCLUSION

The micromechanical forces induced by US energy at a cellular and molecular level exert a wide range of effects on the wound-healing process, including reduction of bacteria within and below the wound bed. Unlike other body cells, bacteria have a rigid cell membrane; repeated pressing of sound waves can disrupt the bacterial membrane, causing cell death.

Besides accelerating the healing speed of open wounds, low frequency US is an effective early treatment for suspected deep-tissue injuries. In vitro and in vivo studies have shown therapeutic efficacies of US techniques in different wounds. However, there is not an exact dose-response for clinical applications of US treatments in different wounds. Considering the promising therapeutic effects of US techniques on the treatment of different wounds, one can expect that US will be a new standard for early treatment of some kinds of wounds. However, to reach such a standard treatment, further studies are required to shed light on the exact mechanism of action and also to provide exact dose-response of therapeutic US for different wounds.

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