REVIEW ARTICLE

A review of mechanism of actions of ultrasound waves for treatment of soft tissue injuries

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Abstract

Objective: Ultrasound (US) waves have unique characteristics that making them promising option for treatment of different soft tissue injuries particularly wounds. The mechanisms of actions of this technique in the treatment of soft tissue are not fully understood. This paper aimed to comprehensively review the biological interactions and mechanism of action of US waves in the treatment of chronic wounds. **Methods:** The databases of PubMed (1990-2016), EMBASE (1990-2016), Web of Sciences (1990-2016), and Google Scholar (1980-2016) were searched using the set terms "US waves" and "wound treatment," "mechanism of action" and "soft tissue injuries," and "biologi8cal interaction." The title and abstract of the collected results were reviewed by two authors, and the relevant papers were selected for further evaluations. **Results:** The mechanisms of action depend on the US physical parameters as well as exposure factors including duration and injury type. The main mechanisms of US waves for wound healing are enhancing the rate of inflammatory phase, stimulating fibroblasts to secrete collagen, enhancing extensibility of collagen, circulation, pain threshold, enzymatic activity, permeability of cell membrane, and accelerating nerve conduction. **Conclusion:** The therapeutic effects of US depend on dose (W/cm^2) and dosage (frequency of application). Low US frequencies show more therapeutic efficiency in wound healing compared with high frequencies.

Key words: Biological interactions, mechanisms of action, soft tissue injuries, treatment, ultrasound wave, wound

INTRODUCTION

During the recent years, different non-medication techniques have been introduced for the treatment of different soft tissue injuries such as pressure relieving beds, cushions, and medicinal plants. They are generally used for prevention and treatment of pressure wounds. In this regard, several physical agent therapies have been developed for the treatment of soft tissue injuries particularly chronic and acute wounds including laser, direct current, electric and magnetic fields, light and electromagnetic fields.[1-6] Some of these methods have received the credit as alternative or adjunctive treatment for some types of wounds.

Ultrasound (US) waves are among the recent methods for treatment of soft tissue injuries with promising outcomes. US-based techniques have unique 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 drug and non-drug techniques.

US waves, because of their unique physical features, have opened their ways in different fields including industrial, environmental, and medical applications. In medical applications, US waves have been investigated for the treatment of several disorders including osteoporosis, malignant tumors, bone fractures, and also wound healing.[7-12]

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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 disorders involving soft tissues.[18-20]

Some applications of high-frequency US include treatment of tendon injuries and relief of the short-term pain.[21-23] Furthermore, US waves can enhance the healing rate of some acute bone fractures, venous and pressure ulcers, and surgical incisions.[21,24,25] However, US treatment may cause burns or can damage the endothelial under inappropriate parameters.[26,27] In line with the research advancements, several commercial machines and modalities have been offered to the market. Most of these machines work at low frequencies and intensities ranging low to moderate. The use of high-frequency US in clinical settings is restricted due to the risk of tissue heating. As a result, considerable research attempts have exploited alternative US parameters. Low-frequency US waves release their energies at low rates which result in low tissue heating. This feature makes the US waves appropriate for healing the slow-to-heal wounds, skin ulcers, and nonunion fractures.

Surface acoustic wave (SAW) patch therapy is another US technique developed for wound treatment. It employs a different acoustic wave than traditional US, utilizing a scattered beam with a maximum penetration of 4 cm, while traditional US can penetrate 10 cm. Some studies have reported increased tissue oxygenation and saturation after the application of SAW patch therapy, which would prove beneficial for wounds or ulcerations being deprived of oxygen and healing factors.[28,29]

US waves have emerged as a promising alternative or adjunctive strategy for chronic wounds. However, the mechanisms of action of these techniques and their biological interactions are not fully understood. In addition, the clinical guidelines on the allowed doses and possible side-effects of these techniques should be determined. Therefore, this paper aimed to comprehensively review the recent advances in the applications of US waves for the treatment of wounds and their biological interactions with human bodies. Furthermore, the recent theories on the mechanisms of action of US waves for the treatment of wounds are discussed.

METHODS

The databases of PubMed (1990-2016), EMBASE (1990-2016), Web of Sciences (1990-2016), and Google Scholar (1990-2016) were searched using the set terms. The search terms included “US wave,” “wound treatment,” “mechanisms of action,” and “biological interactions.” The obtained records were reviewed for the title and abstract by two authors, and they came to consensus whether the studies are related to the review. Animal and human studies in both *in vivo* and *in vitro* designs that evaluate the therapeutic effects and/or mechanisms of action of US waves were included for further evaluations. Any studies that evaluate the effects of US waves on one of the physiological, metabolic, morphological, or physical characteristic of wounds were reviewed. Because of the immense body of literature and variance in the methodology, this study aimed to provide a comprehensive and descriptive overview of the recent advances in applications of US waves for the treatment of wounds and their mechanisms of action and biological interactions with living tissues.

RESULTS

Biological Interactions of US

Wounds are classified into two categories including acute and chronic. The 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 6 weeks, it is considered a chronic wound.[30,31]

Therapeutic US waves are physical method delivering non-ionizing radiation in the form of mechanical sound waves into the tissues to produce heat within the tissue.

High power, high-frequency US is described as US of 0.5-10 MHz and with intensity up to 1500 W/cm^2, whereas low power, low-frequency US is determined as the 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.

Recent *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 US can cause cell death, while low levels US can induce reversible and useful effects.

The “low power” US techniques are used in physiotherapy, fracture healing, 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. There is rigorous evidence in the literature demonstrating that high-intensity US waves can harm bone or delay the bone and tissue healing[32,33] and low intensities can increase the rate of repair and decrease the time of curing.[34,35] Low-intensity pulsed US waves have therapeutic effects on different disorders such as bone fracture healing, osteoporosis, and pain relieving.[36-41]

Clinical evidence shows 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. The 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.[42,43] Although the exact mechanisms of sonophoresis induction are not determined, it is proposed that acoustic cavitation or streaming temporally makes the stratum corneum permeable which consequently increases perfusion.[42-44] 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 the US and various drugs.[43,45]

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. The frequency range of 26 kHz-5 MHz was the most extensively studied range of US for different diseases including wounds.[46] 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.[47]

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

Although the 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.[48-50] The last phase of tissue healing process is "remodeling." In addition, there are some proofs that scar tissue cured with US may be more powerful and elastic than "normal" scar tissue.

The findings of clinical trials, case reports, and observational results have shown that US can increase the rate of various ulcers healing through different mediators.[28,51,52] In addition, in a few cases low-frequency US was also examined to cure burn wounds.[53,54] Furthermore, since the US is identified as a generator for diffusing nitric oxide, it utilizes an auxiliary instrument for vasodilatation and palliation of pain in the treated wound.[55,56]

As the basic understanding of all the therapeutic mechanisms of US improves, treatment regimes are being altered 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."[57] Different systematic reviews of therapeutic US have shown no dose-response relationship.[58,59] However, spatial average temporal average dosage with the range of 0.5-3 W/cm^2 has been reported to minimize adverse effects.[59] Recently, published randomized controlled trials which have reported significant benefits of therapeutic US over placebo US have used dosages of 1-1.5 W/cm^2.[58,60,61]

In clinical experiments, US waves have theragnostic values in different diseases.[5,12] In wound healing applications, both high (1-4 MHz) and low (20-120 KHz) frequencies of US have shown therapeutic outcomes.

The therapeutic effects of US depend on dose (W/cm^2 time) and dosage (frequency of application, series).[62] 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.[62]

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 should permit easy motion of the transducer over the surface of skin.[63]

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 vary to change the dose. At low intensities, both forms produce non-thermal effects.

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.

US and Wound Healing

The US waves are mechanical and transmitted through soft tissues by diffusion and vibration of molecules and are attenuated during passage through the tissue. The intensity of US waves undergoes attenuation because of absorption, scattering or dispersion, reflection, and rarefaction of the wave.[64] 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 0.75-3 MHz where 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[65] 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.[65,66]

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.[67] The larger acoustic impedance difference between two tissues, the less portion of the US wave will transmit through the interface.[68] When reflected US meets further transmitted waves, a standing wave may be generated, which has potential side effects on tissue.[66] 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.

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.[69-71]

Proliferative phase

US techniques have been reported to affect fibroblasts which secrete collagen. Continuous US at higher intensities can heat deeper tissue more effective before 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.[72]

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.[73]

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 ¼ 0.5 W/cm^2, pulsed 2 ms: 8 ms for 5 min).[74]

Mechanisms of Actions 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.[67,75]

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.[76] Extreme thermal effects, which are achieved in high US intensities, may hurt the tissue.[67]

The previous *in vivo* and *in vitro* studies have shown that non-thermal effects of the US such as cavitation and acoustic microstreaming are more significant in the treatment of soft tissue lesions than thermal effects.[77] Cavitation is the formation, oscillation, and collapse of bubbles under the 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.[78] 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 0.1-3 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.[79] The stable cavitation can be suppressed with very short pulses. At least, 1000 cycles at 1 MHz are needed to instate stable cavitation.[79] 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,[68] which has been offered to stimulate tissue repair.[77] Some studies have demonstrated the effects of cavitation and microstreaming *in vitro* experiment such as stimulation of fibroblast repair and collagen synthesis,[14-16,80] regeneration of tissue,[15] and bone healing.[35]

Various mechanisms of action of US in modulating inflamed tissues including increasing the fibrinolysis rate,[17,81] stimulating macrophage-derived fibroblast mitogenic factors,[70] escalating fibroblast recruitment,[69] accelerating angiogenesis,[71] increasing matrix synthesis,[80] synthesizing more dense collagen fibrils,[82] and enhancing tissue tensile strength.[16,83,84] 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.[79] 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.[85,86] Extensive studies of normal and degenerate human tendons have demonstrated striking alteration in composition of matrix,[85-88] 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, 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.[89] Lengthening with thermal doses of US has been shown in the ligament of normal knees[90] and in scar tissue.[91] When the tissue has been heated to an appropriate level which is between 43°C and 45°C,[79] the chance to stretch the tissues lasts for up to 10-min prior the tissue cools.[92]

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 have been shown in some trials[93,94] but not others.[95,96] These studies varied mostly in the applied treatment protocols and regimes. 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.

CONCLUSION

Low-frequency US waves have shown therapeutic efficacies for some types of wounds especially chronic wounds. These mechanical waves can exert therapeutic effects for suspected deep-tissue injuries. *In vitro* and *in vivo* studies have shown therapeutic efficacies of US techniques in different wounds. To reach standard protocols of US waves for wound treatment as well as to develop dosimetric standard for US exposures further controlled clinical trials with high sample size are needed.

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