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Ultrasound and transdermal drug delivery

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Transdermal drug delivery offers an attractive alternative to the conventional drug delivery methods of oral administration and injection. However, the stratum corneum acts as a barrier that limits the penetration of substances through the skin. Application of ultrasound to the skin increases its permeability (sonophoresis) and enables the delivery of various substances into and through the skin. This review presents the main findings in the field of sonophoresis, namely transdermal drug delivery and transdermal monitoring. Particular attention is paid to proposed enhancement mechanisms and future trends in the field of cutaneous vaccination and gene delivery.

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▼ The constantly increasing interest in transdermal delivery derives directly from the advantages of the transdermal route compared with the oral route (Table 1). However, the stratum corneum (the outermost layer of the skin) acts as a barrier to outside invaders entering the human body; it is hence responsible for the poor permeability of skin. This successful role becomes an obstacle to overcome when transdermal drug delivery is desired.

To overcome the main limitation of transdermal delivery (low permeability), innovative technologies have been developed in an attempt to increase transdermal drug delivery as well as to facilitate the extraction of molecules for monitoring and diagnostic purposes [1–3]. These technologies include iontophoresis [4,5], electroporation [6–9], photomechanical waves [10,11] and microneedle array [12,13].

Among the non-invasive methods, low-frequency ultrasound has shown an enhancing effect on the transdermal delivery of various molecules, both *in vitro* and *in vivo*. These methods include: *in vitro* and *in vivo* delivery of insulin [14–16], mannitol [17,18], glucose [18,19] and heparin [20]; *in vivo* delivery of inulin [19]; and the *in vitro* delivery of morphine [21], caffeine [21,22] and lignocaine (lidocaine) [23]. In these studies, the reported enhancement of transdermal transport induced by ultrasound varies from a few percent to several orders of magnitude, depending on the condition.

Ultrasound has also been shown to enhance transdermal transport synergistically with other penetration enhancers, such as chemical enhancers [24,25] and electrical methods [26,27].

Physical characteristics of ultrasound

Ultrasound is defined as sound having a frequency above 18 kHz. Most modern ultrasound devices are based on the piezoelectric effect. This is achieved by applying pressure to quartz crystals and some polycrystalline materials, such as lead–zirconate–titanium or barium titanate, causing electric charges to develop on the outer surface of the material. Thus, application of a rapidly alternating potential across the opposite faces of a piezoelectric crystal will induce corresponding alternating, dimensional changes, thereby converting electrical energy into vibrational (sound) energy [28].

The ultrasound wave is longitudinal in nature (i.e. the direction of propagation is the same as the direction of oscillation). Longitudinal sound waves cause compression and expansion of the medium at a distance of half a wavelength, leading to pressure variations in the medium. The resistance of the medium to the propagation of sound wave is dependent on the acoustic impedance (Z), which is related to the mass density of the medium (ρ) and the speed of propagation (C), according to Equation 1:

$$Z = \rho \times C \qquad \text{[Eqn 1]}$$

The specific acoustic impedances for skin, bone and air are 1.6×10^6, 6.3×10^6 and 400.0 kg/(m^2 s), respectively [28].

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Table 1. Transdermal drug delivery: advantages versus limitations

Advantages	Limitations
No gastrointestinal degradation	Low skin permeability
No first-pass metabolism by the liver	Restricted to potent drugs (low dose achieves the therapeutic effect)
Steady delivery	Cannot deliver large (>500 Da) molecules
Better compliance	Significant lag time
	Skin irritation and/or sensitization

As ultrasound energy penetrates the body tissues, biological effects can be expected to occur if the tissues absorb the energy. The absorption coefficient (a) is used as a measure of the absorption in various tissues. For ultrasound consisting of longitudinal waves with perpendicular incidence on homogeneous tissues, Equation 2 applies:

$$I(x)=I_0\times e^{-ax} \qquad \text{[Eqn 2]}$$

where $I(x)$ is the intensity at depth x, I_0 is the intensity at the surface and a is the absorption coefficient.

To transfer ultrasound energy to the body it is necessary to use a contact medium because of the high impedance of air. The many types of contact media currently available for ultrasound transmission can be broadly classified as oils, water–oil emulsions, aqueous gels and ointments.

There are three distinct sets of ultrasound conditions based on frequency range and applications [29]:

- High-frequency or diagnostic ultrasound in clinical imaging (3–10 MHz).
- Medium-frequency or therapeutic ultrasound in physical therapy (0.7–3.0 MHz).
- Low-frequency or power ultrasound for lithotripsy, cataract emulsification, liposuction, cancer therapy, dental descaling and ultrasonic scalpels (18–100 kHz).

Biological effects of ultrasound

Ultrasound over a wide frequency range has been used in medicine for the past century. For example, therapeutic ultrasound has been used for physical therapy, low-frequency ultrasound has been used in dentistry and high-frequency ultrasound has been used for diagnostic purposes. The utility of ultrasound is continuously expanding and new clinical applications are constantly being developed, including the use of high-intensity focused ultrasound for tumour therapy [30], lithotripsy [31], ultrasound-assisted lipoplasty [32] and ultrasonic surgical instruments [33,34].

Significant attention has thus been given to investigating the effects of ultrasound on biological tissues. Ultrasound affects biological tissues via three main effects: thermal, cavitational and acoustic streaming.

Thermal effects

Absorption of ultrasound increases temperature of the medium. Materials that possess higher ultrasound absorption coefficients, such as bone, experience severe thermal effects compared with muscle tissue, which has a lower absorption coefficient [29]. The increase in the temperature of the medium upon ultrasound exposure at a given frequency varies directly with the ultrasound intensity and exposure time. The absorption coefficient of a medium increases directly with ultrasound frequency resulting in temperature increase.

A recent study [35] suggested the use of a new safety parameter, time to threshold (TT). TT indicates the time after which a threshold temperature rise is exceeded, and how long a piece of tissue can be safely exposed to ultrasound, provided the safe threshold is known.

Cavitational effects

Cavitation is the formation of gaseous cavities in a medium upon ultrasound exposure. The primary cause of cavitation is ultrasound-induced pressure variation in the medium. Cavitation involves either the rapid growth and collapse of a bubble (inertial cavitation), or the slow oscillatory motion of a bubble in an ultrasound field (stable cavitation). Collapse of cavitation bubbles releases a shock wave that can cause structural alteration in the surrounding tissue [36]. Tissues contain air pockets that are trapped in the fibrous structures that act as nuclei for cavitation upon ultrasound exposure. The cavitational effects vary inversely with ultrasound frequency and directly with ultrasound intensity. Cavitation might be important when low-frequency ultrasound is used, gassy fluids are exposed or when small gas-filled spaces are exposed.

Acoustic streaming effects

Acoustic streaming is the development of unidirectional flow currents in fluid that are the result of the presence of sound waves. The primary cause of acoustic streaming is ultrasound reflections and other distortions that occur during wave propagation [37]. Oscillations of cavitation bubbles might also contribute to acoustic streaming. The shear stresses developed by streaming velocities might affect the neighbouring tissue structures. Acoustic streaming might be important when the medium has an acoustic impedance that is different from that of its surroundings, the

fluid in the biological medium is free to move or when continuous wave application is used. The potential clinical value of acoustic streaming has only been minimally explored to date. Nightingale *et al.* [38] used acoustic streaming to help distinguish cystic from solid breast lesions. This study concentrated on detecting the presence or absence of acoustic streaming as an indicator of whether a lesion was cystic or solid. Shi *et al.* [39] used acoustic streaming detection as a tool for distinguishing between liquid blood and clots or soft tissue in haematoma diagnosis.

Effect on skin

Various investigators have reported histological studies of animal skin exposed to ultrasound under various conditions to assess the effect of ultrasound on living skin cells. Levy *et al.* [40] performed histological studies of hairless rat skin exposed to therapeutic ultrasound and reported that application of ultrasound (1 MHz, 2 W/cm^2) induced no damage. Tachibana [41] performed similar studies on rabbit skin exposed to low-frequency ultrasound (105 kHz, 5000 Pa pressure amplitude) and also reported no damage to the skin upon ultrasound application. Mitragotri *et al.* [42,43] performed histological studies of hairless rat skin exposed to low-frequency ultrasound (20 kHz, 12.5–225 mW/cm^2) and found no damage to the epidermis and underlying living tissues. Using scanning electron microscopy, Yamashita *et al.* [44] investigated the effects ultrasound of a frequency of 48 kHz (0.5 W/cm^2) on the surface of hairless mice and human skin. They found that the effect on mice skin was much more significant than on human skin; following ultrasound exposure, the outer layer in mice stratum corneum was totally removed and pores were observed, whereas in human skin some removal of keratinocytes around hair follicles was observed. This effect was attributed mostly to cavitation. Boucaud *et al.* [45] evaluated the effect of low-frequency ultrasound (20 kHz) on hairless mice skin and human skin. Human skin samples that were exposed to low-intensity ultrasound (<2.5 W/cm^2) showed no histological change. Further microscopic examination using transmission electron microscopy confirmed a lack of structural modification.

Based on the above, the effect of ultrasound on skin is derived directly from the application parameters, which include application duration, frequency and intensity.

Use of ultrasound for transdermal drug delivery

In the past two decades, with the development of transdermal delivery as an important means of systemic drug administration, researchers have been investigating the possible application of ultrasound for transdermal delivery systems. Ultrasound has been evaluated at various frequencies in the range of 20 kHz to 16 MHz, and the phenomenon well demonstrated using various molecules [22,46–50].

Therapeutic ultrasound was the most commonly used frequency in early trials [48,51,52]. The ultrasound conditions corresponded to frequencies in the range 0.75–3.00 MHz and an intensity range of 0.0–2.4 W/cm^2 and were chosen initially to avoid potential safety issues. Typical enhancements induced by therapeutic ultrasound are ~10-fold [53]. This enhancement might be sufficient for local delivery of certain drugs, such as hydrocortisone, but not for the systemic delivery of most drugs. Bommanan *et al.* [48,54] hypothesized that, because the absorption coefficient of the skin varies directly with the ultrasound frequency, high-frequency ultrasound energy would concentrate more in the epidermis, thus leading to higher enhancement. To assess this hypothesis, they studied the transport of salicylic acid and lanthanum tracers across hairless rat skin *in vivo* and found that enhancement in the high-frequency range was not significantly higher than in the therapeutic range and therefore not sufficient for the systemic delivery of most drugs.

The effect of low-frequency ultrasound (frequencies below 100 kHz) on transdermal transport has been found to be the strongest. Tachibana [41,55] reported that the use of low-frequency ultrasound (48 kHz) enhanced transdermal transport of insulin across diabetic rat skin. Merino *et al.* [18] compared the enhancing transdermal effect of low (20 kHz) and high (10 MHz) ultrasound and observed significantly increased permeation only for low-frequency ultrasound.

Low-frequency ultrasound has also been used by Mitragotri *et al.* [42,43] to enhance the transport of various low-molecular-weight drugs (including salicylic acid and corticosterone), as well as high-molecular-weight proteins (including insulin, γ-interferon and erythropoeitin), across human cadaver skin *in vitro*. The experimental findings suggest that, among all the ultrasound-related phenomena evaluated (cavitation, thermal effects, generation of convective velocities and mechanical effects), cavitation plays the dominant role in sonophoresis, suggesting that application of low-frequency ultrasound should enhance transdermal transport more effectively. Mitragotri *et al.* [43] found that the enhancement induced by low-frequency ultrasound is up to 1000-fold higher than that induced by therapeutic ultrasound. For example, application of ultrasound (20 kHz, 225 mW/cm^2, 100-ms pulses applied every second) to a chamber glued onto the back of the rat back and filled with insulin solution (100 U/ml) reduced the blood glucose level of diabetic hairless rats from ~400 to 200 mg/dl in 30 min [42].

Recently, Lee *et al.* [56] studied the use of short ultrasound exposure time to deliver insulin to hyperglycaemic rats. They used a lightweight cymbal array at 20 kHz and 100 mW/cm^2. For the 10- and 5-min ultrasound exposure groups, the glucose concentration decreased from the baseline to –174.6 ± 67.2 and –200.4 ± 43.4 mg/dl, respectively, measured after 1 h. These results indicate that ultrasound exposure time need not be long to deliver a therapeutic insulin dose.

In the early studies, ultrasound was applied together with the drug to enhance the transport process through its convective effect. In more recent studies, Mitragotri and Kost [57] found that a short application of ultrasound could increase skin permeability for a prolonged period of time. Ultrasound at a frequency of 20 kHz and intensity of 7 W/cm^2 was applied only once to each animal for less than 2 min to increase the skin permeability. The diffusion of several molecules after ultrasound pretreatment of the skin was evaluated. For example, the enhanced diffusion of mannitol (MW of 180) and inulin (MW of 5000) after ultrasound pretreatment of the skin was found to be 20-fold higher for inulin and 33-fold higher for mannitol. Based on the enhanced inulin skin permeability, the authors predict that a typical baseline insulin (comparable in size to inulin) dose of ~1 U/h can be delivered through a patch having an area of 10 cm^2 and containing insulin solution at a concentration of 500 U/ml. This dose can be further increased by providing an additional driving force, such as the application of lower-intensity ultrasound during transport to modulate the insulin delivery rates.

Katz *et al.* [58] used pretreatment with low-frequency ultrasound (55 kHz, 12 W) to shorten the lag-time for analgesic agent [EMLA® cream (AstraZeneca; http://www.astrazeneca.com)] to be effective. EMLA cream is a mixture of two local anaesthetics (lignocaine and prilocaine). It is indicated for use on normal intact skin to induce local analgesia about 60 min after application. The study was conducted on 42 human subjects and pain score and patient preference were measured. After ultrasound pretreatment (4–14 s) and then 5, 10 and 15 min after EMLA cream application, pain scores and overall preference were statistically indistinguishable from EMLA cream application for 60 min. These results suggest the potential for rapid analgesia for painful cutaneous procedures.

Transdermal monitoring using ultrasound

Considerable effort has been directed towards developing painless and convenient methods to measure blood analytes, particularly glucose, including implantable sensors, minimally invasive skin microporation, approaches involving laser or miniaturized lancets and noninvasive technologies such as near-infrared spectroscopy, transdermal permeation enhancers and reverse iontophoresis. One of the fundamental problems in noninvasive transdermal diagnostics is obtaining sufficient quantities of analyte for detection. Ultrasound, particularly at low frequencies, has been shown to increase skin permeability, hence allowing sufficient amounts of clinically relevant analytes, including glucose, to be collected for the purpose of noninvasive monitoring [1,3].

The technique was assessed on type 1 diabetic volunteers to determine whether a single short application of ultrasound (less than 2 min) was sufficient to extract glucose noninvasively across human skin for several hours, and to determine whether transdermal glucose flux varied in response to variations in blood glucose concentrations. Additional experiments to further assess the duration of ultrasound-induced permeability found that the skin permeability remained high for about 15 h and decreased to its normal value by 24 h. A comparison of venous blood glucose levels and noninvasively extracted glucose fluxes after ultrasound pretreatment showed close correlation. Site-to-site variability of skin permeability after ultrasound application was also evaluated within the same patient and between patients. The site-to-site variability was about the same as patient-to-patient variability. This indicates the necessity of one-point calibration between transdermal glucose flux and one blood sample, which can then be used to predict subsequent blood glucose values. Based on such a calibration, the correlation was assessed between transdermal glucose flux and blood glucose values (mean relative error of 17%). Although further studies to assess safety (particularly the effect of repeated extractions) will be required, the initial safety studies indicated that ultrasound did not induce adverse effects on the skin, no damage or irritation being observed by visual inspections. The possibility of using ultrasound to enhance transdermal transport of diverse substances of wide-ranging molecular size and chemical composition could be useful in both diagnostics and drug delivery. The results are especially encouraging given that the ultrasound device used in this study [VCX400 (Sonics and Materials; http://www.sonicsandmaterials.com)] was not designed or optimized for this application.

Sontra Medical (http://www.sontra.com) has been developing this technology for noninvasive continuous sensing of glucose and for enhanced topical anaesthesia. Recently, a minimally invasive system that continuously measures glucose flux through ultrasonically permeated skin was reported [59]. In this study, the glucose level of ten diabetes patients were monitored over a period of 8 h. Generally speaking, a good correlation was observed between sensor

output reading and blood glucose measurements. The investigators recognized several areas where improvements should be made to increase sensitivity, reduce variations and increase accuracy.

Mechanism

The mechanism of improved transdermal transport by ultrasound has been studied for the past 20 years. In spite of the large number of studies that have been published, the mechanism is still not well understood or characterized. A possible mechanism of improved percutaneous transport by ultrasound suggested by several groups [40,51,60] is that ultrasound might interact with the structural lipids located in the intercellular channels of the stratum corneum. This is similar to the postulated effects of some chemical transdermal enhancers that act by disordering lipids. Tachibana [41] and Simonin [61] postulated that the energy of ultrasonic vibration enhanced transdermal permeability through the transfollicular and transepidermal routes, suggesting that microscopic bubbles (cavitation) produced at the surface of the skin by ultrasonic vibration might generate a rapid liquid flow, thereby increasing skin permeability.

Mitragotri *et al.* [43] evaluated the role played by various ultrasound-related phenomena, including cavitation, thermal effects, generation of convective velocities and mechanical effects. The authors hypothesized that transdermal transport during low-frequency ultrasound application occurs across the keratinocytes rather than the hair follicles. They suggested that cavitation causes disorder of the stratum corneum lipids, resulting in significant water penetration into the disordered lipid region. This might cause the formation of aqueous channels through the intercellular lipids of the stratum corneum through which permeants could move. Tang *et al.* [62] studied the relative roles of enhanced diffusion due to ultrasound-induced skin alteration and enhancement due to ultrasound forced convection. The findings (theoretical and experimental) suggest that, for low-frequency ultrasound, the relative contribution depends on the *in vitro* skin model studied. Specifically, convection plays an important role when heat-stripped stratum corneum is exposed to ultrasound, whereas its effect is minimal when full-thickness skin is utilized. In addition, the effective pore radius of the skin estimated using heat-stripped stratum corneum during ultrasound exposure is much larger than that within full-thickness skin.

All recent studies indicate that cavitation plays an important role in the enhancing mechanism. Several attempts have been made to establish a suitable mathematical model that will describe the enhancement phenomenon and predict the enhancement ratio for different drugs in various conditions [61–63].

Tezel and Mitragotri [64] describe a theoretical analysis of the interaction of cavitation bubbles with the stratum corneum lipid bilayers. Three modes were evaluated – shock-wave emission, microjet penetration into the stratum corneum and impact of microjet on the stratum corneum. Their suggested model predicts that both microjets and spherical collapses might be responsible for the enhancement effect.

Synergistic effects of ultrasound

Ultrasound might enhance transdermal transport by inducing skin alteration, as well as by inducing active transport (forced convection) in the skin. Various other means of transport enhancement, including chemicals [24,52,65,66], iontophoresis [27] and electroporation [26], might enhance transport synergistically with ultrasound. Mitragotri *et al.* [25] evaluated the synergistic effect of low-frequency ultrasound with chemical enhancers and surfactants, including sodium lauryl sulfate (SLS) and a model permeant, mannitol. Application of ultrasound alone as well as SLS alone, both for 90 min, increased skin permeability about threefold for SLS and eightfold for ultrasound. However, combined application of ultrasound and 1% SLS solution induced an increase in skin permeability to mannitol in the order of 200-fold.

Ultrasound also exhibited a synergistic effect with electroporation [26]. Ultrasound reduced the threshold voltage for electroporation as well as increasing transdermal transport at a given electroporation voltage. The enhancement of transdermal transport induced by the combination of ultrasound and electroporation was higher than the sum of the enhancement induced by each enhancer alone.

Combined application of ultrasound and iontophoresis also has practical implications. The combination of ultrasound and electric current offers a higher enhancement than that offered by each of them individually under the same conditions. Since ultrasonic pretreatment reduces skin resistivity, a lower voltage is required to deliver a given current during iontophoresis compared to that in controls. This should result in lower power requirements as well as possibly less skin irritation [27].

Future trends

Vaccination

In recent years, the potential for exploiting the skin for purposes of vaccination has received a great deal of attention [67–72].

Transcutaneous immunization provides access to the immune system of the skin, which is dominated by densely

distributed and potent antigen-presenting cells (Langerhans cells). Langerhans cells have been shown to play essential roles in the induction of T-cell-mediated immune reactions against a wide variety of antigens [69,73]. In order for this technique to be practical, the vaccine, which is generally a large molecule or complex, has to penetrate the stratum corneum barrier. Normally, skin is not permeable under these conditions. One common strategy is to use an adjuvant, which is a compound used to enhance the immune response to vaccine compounds. Glenn *et al.* [74] found that applying cholera toxin to the surface of the skin stimulates an immune response to vaccine compounds such as diphtheria or tetanus toxoids. Another strategy is to use physical enhancers such as ultrasound. Ultrasound can be used to enhance skin permeability to both the adjuvant and the vaccine, and hence to facilitate their delivery to the target cells.

Gene therapy

Another future application for ultrasound as a topical enhancer, which seems to show promise, lies in the field of topical gene therapy [75,76]. Gene therapy is a technique for correcting defective genes that are responsible for disease development, most commonly by replacing an 'abnormal' disease-causing gene with the 'normal' gene. A carrier molecule (vector) is usually used to deliver the therapeutic gene to the target cell. Topical delivery of the vector–gene complex can be used for target cells within the skin, as well as for the systemic circulation. The identification of genes responsible for almost 100 diseases affecting the skin has raised the option of using cutaneous gene therapy as a therapeutic method [77]. The most obvious candidate diseases for cutaneous gene therapy are the severe forms of particular genodermatoses (monogenic skin disorders), such as epidermolysis bullosa and ichthyosis. Other applications might be healing of cutaneous wounds such as severe burns and skin wounds of diabetic origin [78].

Topical gene therapy acquires the penetration of a large complex to or through the skin. Ultrasound pretreatment of the skin will increase its permeability and permit the delivery of the carrying vector.

Safety

The utility of ultrasound in medicine as a technical tool, as well as a therapeutic agent, is constantly increasing. In view of this, much concern is directed to the issues of ultrasound bioeffects and safety. The World Federation for Ultrasound in Medicine and Biology (WFUMB; http://www.wfumb.org) has issued several publications related to safety of ultrasound bioeffects, addressing specifically thermal bioeffects [79] and nonthermal bioeffects [80] in an attempt to reach an international consensus and to adopt a policy on safety guidelines. The use of ultrasound as an aid to increasing skin permeability is based on its nonthermal bioeffects, mostly cavitation. In view of this, much attention should be paid to the issue of ultrasound affecting the structure of the skin: is it a reversible or irreversible change? What is the role of the free radicals that are generated during the cavitation process within the skin?

To develop a useful tool based on ultrasound technology, further research focusing on safety issues is required to evaluate limiting ultrasound parameters for safe exposure.

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