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Review

Sonophoresis: recent advancements and future trends

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Abstract

Objectives Use of ultrasound in therapeutics and drug delivery has gained importance in recent years, evident by the increase in patents filed and new commercial devices launched. The present review discusses new advancements in sonophoretic drug delivery in the last two decades, and highlights important challenges still to be met to make this technology of more use in the alleviation of diseases.

Key findings Phonophoretic research often suffers from poor calibration in terms of the amount of ultrasound energy emitted, and therefore current research must focus on safety of exposure to ultrasound and miniaturization of devices in order to make this technology a commercial reality. More research is needed to identify the role of various parameters influencing sonophoresis so that the process can be optimized. Establishment of long-term safety issues, broadening the range of drugs that can be delivered through this system, and reduction in the cost of delivery are issues still to be addressed.

Summary Sonophoresis (phonophoresis) has been shown to increase skin permeability to various low and high molecular weight drugs, including insulin and heparin. However, its therapeutic value is still being evaluated. Some obstacles in transdermal sonophoresis can be overcome by combination with other physical and chemical enhancement techniques. This review describes recent advancements in equipment and devices for phonophoresis, new formulations tried in sonophoresis, synergistic effects with techniques such as chemical enhancers, iontophoresis and electroporation, as well as the growing use of ultrasound in areas such as cancer therapy, cardiovascular disorders, temporary modification of the blood–brain barrier for delivery of imaging and therapeutic agents, hormone replacement therapy, sports medicine, gene therapy and nanotechnology. This review also lists patents pertaining to the formulations and techniques used in sonophoretic drug delivery.

Keywords drug delivery; phonophoresis; sonophoresis; transdermal; ultrasound

Introduction

The skin has been investigated as route for drug administration for several decades and many drug delivery techniques that use alternative forms of energy to facilitate permeation of drugs across the skin have been explored. Sonophoresis describes the use of ultrasound to move low and high molecular weight drugs through intact living skin and into the soft tissues.[1,2] It is one of the most promising novel drug delivery system and has been shown to enhance the skin penetration and release rate of a number of drugs that have poor absorption/permeation profiles through the skin.[3–6]

Sonophoresis is a localised, non-invasive, convenient and rapid method of delivering low molecular weight drugs as well as macromolecules into the skin,[7] and has been widely reviewed.[4,7,8–14] These reviews summarised various aspects relating to sonophoresis, overviewed applications, mechanisms, factors that influence sonophoretic drug delivery, clinical studies, synergistic effect of ultrasound with chemical enhancers and iontophoresis and the biological effects of ultrasound; however, some important considerations were overlooked, such as insight into various patents filed, commercial devices launched and effects of formulations. No significant critical review on sonophoresis has been found in the literature since 2004, although the mechanistic principles and current status of sonophoresis under low-frequency conditions were discussed in detail in a recent theme issue 'Ultrasound and gene delivery' in *Advanced Drug Delivery Reviews*.[15]

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Sonophoresis provides the usual advantages of a transdermal route, such as improved therapeutic efficacy by bypassing hepatic first-pass metabolism, and avoiding the inconvenience associated with parenteral drug delivery and the variation in absorption that occurs with oral administration.[2,16,17] In addition, it reduces the chance of dosing variation by providing programmed delivery of the drug.[18] Sonophoresis also provides a therapeutic regimen that improves patient compliance. It permits the use of a drug with a short biological half-life, since the drug is delivered to the target area without the need to recirculate in the blood. Moreover, the drug is delivered into the blood stream directly without any delay. It also allows for rapid termination of the effect by turning off the sonophoretic system.[19–24] Thus, given the many advantages associated with this system, it has been an area of growing interest in the local and the systemic delivery of various drugs.[1,25,26]

Enhancement of drug delivery is determined by various parameters, including frequency, intensity, duty cycle and application time.[27–32] Low-frequency ultrasound (20 kHz) has been found to be more potent in enhancing skin permeability than therapeutic ultrasound (1–3 MHz).[3] This has been attributed to the phenomena of cavitation, which contributes most to drug permeation enhancement but is difficult to generate at high frequencies.[33] However, this paper failed to explain the increased permeability under low-frequency conditions. Similarly, a direct relationship between intensity and increase in permeation has been established, but the rate of permeation does not continue to increase with increasing intensity. An extensive literature search did not discern any correlation between ultrasound frequency, intensity, molecular structure, duty cycle, application time and the degree of enhancement. However, this may reflect the wide range of drugs used, different experimental conditions, animal models, membranes selected and end points used in evaluating techniques.

Enhancement of drug penetration in the skin by phonophoresis is suggested to be due to its thermal and mechanical effects – inertial cavitation, acoustic streaming and generation of convective velocities.[34–37] Ultrasound-enhanced transdermal transport is mediated by inertial cavitation, where collapses of cavitation bubbles microscopically disrupt the lipid bilayer of the stratum corneum.[38] Recently, Lavon and colleagues showed that bubble growth within the skin due to rectified diffusion may play a significant role in sonophoresis.[39]

Phonophoresis is divided into low-frequency (<1 MHz), therapeutic (1–3 MHz) and high-frequency (3–15 MHz) phonophoresis. Most studies and clinical applications are within these ranges.[40–42] Phonophoresis has been used clinically to assist in the permeation of various drugs.[5] Therapeutic ultrasound can enhance the transdermal permeation of low-molecular-weight drugs, and it has been reported that low-frequency ultrasound of 0.02–0.2 MHz generates significant energy and allows the deep transdermal permeation of drugs that are difficult to permeate at therapeutic frequencies.[14] This indicates that sonophoresis is indeed a reality for such molecules under specific conditions.

Ultrasound therapies are widely used in physiotherapy. Apart from this, therapeutic ultrasound is used currently in research in sonoporation,[43] gene therapy,[44] bone healing,[45] sonothrombolysis,[46] and sports medicine,[47,48] which are described in more detail later in this review. This review looks at the advances in this field, focusing on recent developments, the current status and the opportunities that transdermal sonophoresis offers in this new millennium. Table 1 lists patents relating to this technology that have been filed.[49–83]

Equipment and devices

Ultrasound waves are created when a generator produces electrical energy that is converted to mechanical energy through the deformation of piezoelectric material in a transducer.[84] The waves produced are transmitted by propagation through molecular oscillations in biological tissue.[85] The piezoelectric material can be lead zirconate titanate, polyvinyl fluoride, thin-film zinc oxide, lead titanate or the piezo-ceramic/polymer composites, lead metaniobate, barium titanate or modified lead titanate.[86] Sonicators operating at various frequencies in the range of 20 kHz to 3 MHz that can be used for sonophoresis are available commercially.[87] The design and construction of portable, efficient and cost-effective devices is currently a thriving area of research in sonophoresis.

Maione and colleagues focused their research on the design and construction of a small lightweight transducer or array. To obtain the desired intensity range, a cymbal transducer design was chosen because of its light, compact structure and low resonance frequency in water. In order to increase the spatial ultrasound field for drug delivery across skin, two arrays, each comprising four cymbal transducers, were constructed.[88]

Smith and colleagues explored the feasibility of using ultrasound by novel transducers for enhancing the transport of insulin across skin *in vitro*. They also explored the use of the cymbal transducer as both a single element and configured as an array for transdermal insulin delivery, and accurately quantified the acoustic field.[89]

Yeo and Zhang developed and investigated a new sonophoresis device with dual flat flextensional ultrasound transducers.[90] This device has a radiated acoustic intensity about 2–4 times higher than that generated by a single ultrasound transducer. The device has the capability to reduce the applied voltage at least twofold. The proposed sonophoresis devices with double ultrasound transducers weighs only 73.3 g; by comparison the ultrasonic probe from a commercial sonicator weighs about 1 kg. The authors also proposed the new concept of a highly compact sonophoresis microdevice to overcome some of the drawbacks of commercial equipment.

Several types of sonophoresis devices have been developed in recent years. Lee and colleagues demonstrated the feasibility of using short ultrasound exposure times to non-invasively deliver insulin using a lightweight (<22 g), low-profile ($37 \times 37 \times 7$ mm^3) cymbal array (f = 20 kHz).[91] Their results indicated that ultrasound exposure times do not need to be long to deliver a clinically significant insulin dose that reduces high blood glucose.

Table 1 Patents relating to sonophoresis

Title of patent	US patent no.
Topical application of medication by ultrasound with coupling agent	4309989[49]
Disposable piezoelectric polymer bandage for percutaneous delivery of drug and method for such percutaneous delivery	4787888[50]
Ultrasound enhancement of transdermal drug delivery	4767402[51]
Ultrasound enhancement of membrane permeability	4780212[52]
Ultrasound enhancement of transbuccal drug delivery	4948587[53]
Local application of medication with ultrasound	5016615[54]
Ultrasound-enhanced delivery of materials into and through the skin	5115805[55]
Drug delivery by multiple frequency phonophoresis	5267985[56]
Ultrasound-enhanced delivery of materials into and through the skin	5231975[57]
Ultrasound-enhanced delivery of materials into and through the skin	5323769[58]
Method for enhancing delivery of chemotherapy employing high frequency force fields	5386837[59]
Enhancement of transdermal delivery with ultrasound and chemical enhancers	5445611[60]
Ultrasonic transdermal drug delivery system	5421816[61]
Enhancement of transdermal monitoring applications with ultrasound and chemical enhancers	5458140[62]
Sonophoretic drug delivery system	5656016[63]
Ultrasonic method and apparatus for cosmetic and dermatological applications	5618275[64]
Enhancement of transdermal monitoring applications with ultrasound and chemical enhancers	5722397[65]
Transdermal protein delivery using low frequency sonophoresis	6002961[66]
Chemical and physical enhancers and ultrasound for transdermal drug delivery	5947921[67]
Effect of electric field and ultrasound for transdermal drug delivery	6041253[68]
Transdermal protein delivery or measurement using low-frequency sonophoresis	6018678[69]
Method and apparatus for therapeutic treatment of skin with ultrasound	6113559[70]
Ultrasound enhancement of percutaneous drug absorption	6030374[71]
Sonophoresis method and apparatus	6322532[72]
Sonophoretic enhanced transdermal transport	6190315[73]
Ultrasound enhanced chemotherapy	6308714[74]
Ultrasound enhancement of percutaneous drug absorption	6398753[75]
Ultrasound enhancement of transdermal transport	6491657[76]
Method and apparatus for in-vivo transdermal and/or intradermal delivery of drugs by sonoporation	6487447[77]
Method and apparatus for producing homogenous cavitation to enhance transdermal transport	6620123[78]
Sonophoresis apparatus	European Patent 1089788[79]
Device for a transdermal and phonophoretic combination therapy and the use thereof in a method for medical application	6868286[80]
Method and apparatus for in-vivo transdermal and/or intradermal delivery of drugs by sonoporation	6842641[81]
Ultrasound enhancement of percutaneous drug absorption	7004933[82]
Ultrasound mediated transcleral drug delivery	Wipo Patent WO/2007/081750[83]

Several different low-frequency transducer designs can be used for drug delivery, such as low-frequency flextensional resonators,[92] tonpliz transducers,[93] and 'thickness'-type resonators.[94] A recent comprehensive review on ultrasound drug delivery commented on the need to develop small low-frequency transducers that patients can wear.[95]

Luis and colleagues found that circular cymbal ultrasound arrays were effective in delivering therapeutic levels of insulin in rats, rabbits and pigs.[96] However, a rectangular cymbal design, desired in order to achieve a broader spatial intensity field without increasing the size of the device or the spatial-peak temporal-peak intensity, improved the efficiency of drug delivery.

Park and colleagues investigated the feasibility of a lightweight cymbal transducer array as a practical device for non-invasive transdermal insulin delivery in large pigs.[97] Their findings indicated the feasibility of ultrasound-mediated transdermal insulin delivery using the cymbal transducer array in animals of similar size and weight to humans.

The literature reported here illustrates major advancements in the field of miniaturisation, since the availability of easy-to-use devices has been a significant hurdle to the adoption of low-frequency sonophoresis in clinical medicine.

Ultrasound–tissue interaction

The three major factors that govern sonophoretic drug delivery are the physicochemical properties of the drug formulation, the ultrasound parameters and the skin (Figure 1).[98] Sonophoretic drug delivery is likely to be influenced by the structure and physiological changes in the skin, the vehicle used to deliver the drug, and the quantum of energy and the duration for which this energy is provided. Though the structure and physicochemical properties of the drug will influence the permeation rate, it will be assumed that this delivery system will not be limiting itself for a particular category of the drug. As the ultrasound energy interacts with

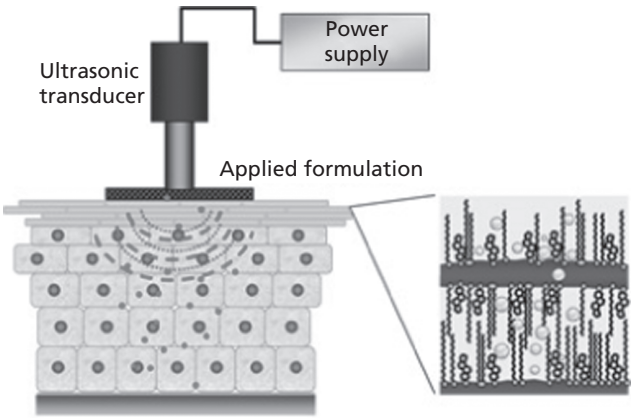

Figure 1 Sonophonetic drug delivery. Drug is placed on the skin beneath the ultrasonic probe. Ultrasound pulses are passed through the probe, and it is hypothesised that drug molecules move into the skin by a combination of physical wave pressure and permeabilisation of intercellular bilayers.[98]

the tissue it traverses, it will be encounter changes in density, pH and chemical constituents in the different layers. These changes are not only mechanical effects but also relate to electrical conductivity, sonochemical changes and thermal effects. Since ultrasound is a form of energy, it will undoubtedly interact with the viable as well as dead layers of the skin. Histopathological studies are the best way to explore configurational changes in skin layers after ultrasound treatment.

Sonophoretic enhancement has been observed and explained by several investigators over the years.[4,9,11,12,14] They have suggested various hypotheses, but no conclusive evidence has been put forward. The answer to the question as to how ultrasound modifies the tissue with which it is interacting cannot be given by one single mechanism because many different potentially modulating physical situations are generated simultaneously by the ultrasonic wave, and the theoretical and experimental basis of the intricate mechanism is in its infancy. The complexity of the skin structure and the changes in tissue property *vis á vis* ultrasound inputs play important roles in transdermal drug delivery. Structural changes in the skin barrier layer are the result of various acoustic phenomena taking place at the skin–transducer junction, such as refraction, reflection, absorption and scattering. This leads to cause–effect phenomena like perturbation of biomembrane lipid–protein configurations, bubble formation, cavitation and even microstreaming after long exposures at high intensities of ultrasound exposure.

Thermal effects

Ultrasound cannot propagate through tissue without some of its associated energy being deposited as heat. This heat will result in increased temperature of the tissue if its rate of input exceeds the capacity of that tissue to dissipate it. Thermal effects are important with high-intensity continuous-wave ultrasound and are prominent when the irradiated tissue has high protein content or includes bony regions, and when the vascular supply to the area is poor.[99,100]

Cavitation

Cavitation is the result of the pressure changes associated with the propagation of a compressional wave (which is the only wave that can propagate for large distances through soft tissues). This may lead to structural disordering of the stratum corneum lipids, due to oscillations of the ultrasound-induced cavitation bubbles near the keratinocyte lipid bilayer interfaces. Cavitation bubbles also generate shock waves upon collapse and this may also contribute to the structure-disordering effect. The diffusion of permeants through a disordered bilayer phase would naturally be higher than that through normal bilayers.[38]

Streaming effect

The streaming effect becomes more important when continuous-wave application is used and the fluid is free to move in a biological medium whose acoustic impedance is different from its surroundings. These mechanisms are illustrated in Figure 1, which shows the basic design of an ultrasonic device.[87]

Numerous mechanical effects occur when the energy density of an ultrasonic wave exceeds a certain threshold value. The rate at which ultrasonic energy is supplied to the tissue, that is, the intensity of the beam (which is one of the main parameters) appears to determine the biological effects that will result from that exposure.

Formulation – the crucial link between drug and device

Though the transdermal route is more suitable for lipophilic drugs and poses a resistance for hydrophilic drugs, ultrasound-mediated delivery is better for hydrophilic drugs. These drugs should be formulated in such a manner that they can be dissolved, dispersed or distributed in the coupling medium, or the formulation itself acts as a coupling medium (to ensure proper contact between the transducer and the skin). A literature survey reveals that a wide variety of formulations have been used in sonophoretic studies: solutions, gels, ointments, creams, liposomes, solid lipid microparticles, microspheres, matrices and occlusive dressings. The results of sonophoretic studies for drug delivery are summarised in Table 2.[101–118]

Gel formulations

The importance of vehicular effects has been demonstrated in experiments where hairless mice were immersed in either lidocaine gel or aqueous lidocaine solution and exposed to 0.048 MHz ultrasound at 0.17 W/cm^2.[119] Application of ultrasound under these conditions prolonged the anaesthetic effect of lidocaine.

Yang and colleagues carried out a study to determine the feasibility of using gel formulations for the transdermal delivery of the synthetic glucocorticoid triamcinolone acetonide (TA) in conjunction with phonophoresis to develop carbopol TA gels.[120] The anti-inflammatory effects of the TA-containing gel after the absorption of ultrasound were evaluated by measuring changes in serum creatine phosphokinase *in vivo*, and histological findings. They concluded

Table 2 Drugs used in sonophoresis in recent years

Drug	Membrane used	Experimental conditions	Results	References
Fentanyl (F) & caffeine (C)	Human skin, *in vitro*	Fen: 20 kHz, 2.5 W/cm^2, P (60 min) Caf: 20 kHz, 2.5 W/cm^2, P (60 min) Fen: 20 kHz, 2.5 W/cm^2, C (60 min) Caf: 20 kHz, 2.5 W/cm^2, C (60 min)	× 34 enhancement × 4 enhancement × 4 enhancement × 1 enhancement	Boucaud *et al.* 2001[101]
Heparin	Pig skin, *in vitro*	20 kHz, 7 W/cm^2, P (10 min)	× 21 enhancement	Mitragotri & Kost 2001[102]
Caffeine & morphine	Hairless mouse skin, *in vitro*	Caf: 40 kHz, 0.44 W/cm^2, C (4 h) Mor: 40 kHz, 0.44 W/cm^2, C (4 h)	× 4 enhancement × 10 enhancement	Monti *et al.* 2001[103]
Dalteparin	Rats, *in vivo*	20 kHz, 2.5 W/cm^2, P (15 min)	Anti-Xa activity	Mitragotri & Kost 2001[102]
Atenolol (Ate), carteolol (Car), timolol (Tim), betaxolol (Bet)	Rabbit eyes, *in vitro*	Ate: 20 kHz, 2 W/cm^2 (60 min) Car: 20 kHz, 2 W/cm^2 (60 min) Tim: 20 kHz, 2 W/cm^2 (60 min) Bet: 20 kHz, 2 W/cm^2 (60 min)	× 2.6 enhancement × 2.8 enhancement × 1.9 enhancement × 4.4 enhancement	Zderic *et al.* 2002[104]
Mannitol	Pig skin, *in vitro*	20 kHz, 1.6–14 W/cm^2, P (90 min)	× 10 enhancement	Mitragotri *et al.* 2000[105]
Insulin	Rats, *in vivo*	20 kHz, 2.5 W/cm^2, P (15 min)	Marked decrease in glucose levels	Boucaud *et al.* 2001[101]
EMLA cream	42 human subjects	55 kHz, (60 min), Power 13 W RMS	Onset of at least superficial cutaneous analgesic achieved as fast as 5 min	Katz *et al.* 2004[106]
Lidocaine	32 healthy male volunteers	0.5 and 1 MHz, 2 W/cm^2, C	Surface anaesthesia phonophoresis group showed a significantly higher pain threshold than other groups	Kim *et al.* 2007[107]
Ciclosporin A	Rat skin, *in vitro*	20 kHz, 0.4, 0.8 and 1.2 W/cm^2, P (30 min)	7-fold increase in concentration of drug in skin	Liu *et al.* 2006[108]
Oligonucleotides	Full-thickness pig skin, *in vitro*	20 kHz, 2.4 W/cm^2, P (10 min)	Successful delivery of antisense oligonucleotides	Tezel *et al.* 2004[109]
Piroxicam	Hairless mouse skin, *in vitro* and *in vivo*	1 and 3 MHz, 1, 1.5 and 2 W/cm^2, C & P (10 h)	Highest permeation observed at 1 MHz, 2.0 W/cm^2, continuous output	Chung *et al.* 2002[110]
Ibuprofen	60 osteoarthritis patients (target knee joint)	1 MHz, 1 W/cm^2 (5 min)	Ibuprofen phonophoresis found to be effective and generally well tolerated after 10 therapy sessions but it was not superior to conventional ultrasound in patients with knee osteoarthritis	Kozanoglu *et al.* 2003[111]
Caffeine	Swine dorsal region, *in vitro*	3 MHz, 0.2 W/cm^2, C (1 min/cm^2)	ultrasound as effective as accentuater and accelerator of cutaneous caffeine permeation	Campos *et al.* 2007[112]
Dexamethasone	10 healthy subjects	3 MHz, 1.0 W/cm^2, P (5 min)	Phonophoretic effect occurred with drug when its application saturated the skin	Saliba *et al.* 2007[113]
Calcein & D_2O	Excised hairless rat skin, *in vitro*	41–445 kHz, 60–240 mW/cm^2 (30 min)	Calcein: 41 kHz: × 22.3 enhancement 158 kHz: × 6.3 enhancement 445 KHz: × 3.8 enhancement D_2O 41 KHz: × 55 enhancement	Mutoh *et al.* 2003[114]
Sulforhodamine B	Pig skin, *in vitro*	19.6, 36.9, 58.9, 76.6 and 93.4 kHz, 0.54 W/cm^2, C (15 min)	For each frequency applied, there was a threshold intensity below which no enhancement was observed; this intensity increased with frequency	Tezel *et al.* 2001[33]
Sulforhodamine B	Full-thickness pig skin, *in vitro*	20 kHz, 7.5 W/cm^2, P (10 min)	Ultrasound enhances surfactant delivery and dispersion	Tezel *et al.* 2002[115]
Nile red & calcein	Full-thickness porcine skin, *in vitro*	20 kHz, 15 W/cm^2, P (2 h)	Lipid removal from stratum corneum implicated as factor contributing to observed permeation enhancement effects of low-frequency ultrasound	Alvarez-Roman *et al.* 2003[116]
Sodium fluorescein	Rabbit eyes, *in vitro*	880 kHz, 0.19, 0.34 and 0.56 W/cm^2, P (5 min)	× 10 enhancement	Zderic *et al.* 2004[117]
Testosterone	Rat abdomen skin, *in vitro*	1 MHz, 0.5 W/cm^2, C (1 h) and 20 kHz, 2.5, 3.25 and 5 W/cm^2, P (30 min)	Low-frequency ultrasound resulted in higher transdermal permeation than high-frequency	El-Kamel *et al.* 2008[118]

C, continuous mode; P, pulsed mode.

that a TA gel using phonophoresis might be used as a new transdermal delivery technique providing enhanced anti-inflammatory effects.

Campos and colleagues studied the influence of ultrasound in cutaneous permeation of caffeine: 5% caffeine gel plus ultrasound treatment was given to skin extracted from swine dorsal region.[112] It was concluded that ultrasound was effective as an accentuator and accelerator of cutaneous caffeine permeation.

Kim and colleagues looked at the anaesthetic effects of 5 g lidocaine hydrochloride gel using low-frequency ultrasound (0.5 and 1 MHz), which was applied to the wrists of healthy volunteers after applying a commercial ultrasound gel.[107] In terms of surface anaesthesia, the groups exposed to ultrasound showed a significantly higher pain threshold than the groups not exposed to ultrasound. In addition, it was found that deep penetration of lidocaine improved the anaesthetic effect.

Ointments and creams

Asano and colleagues studied the effect of pulsed-output ultrasound (1 MHz) with on : off ratios of 1 : 2, 1 : 4 and 1 : 9 on the transdermal absorption of indometacin from an ointment in rats.[121] Ultrasound energy was applied for 10–19 min at a range of intensities (1.0–2.5 W/cm^2), energy levels commonly used for therapeutic purposes. The on : off pulsed ratio, intensity and the time of application all influenced the transdermal phonophoretic delivery system of indometacin; 1 : 2 pulsed-output ultrasound appeared to be the most effective in improving transdermal absorption. The highest penetration was observed at an intensity of 1.0 W/cm^2 and application time of 15 min. Pulsed output enabled use of higher intensities of ultrasound without increasing skin temperature or damaging the skin.

Kost and colleagues studied the onset and efficiency of cutaneous anaesthesia provided by EMLA (eutectic mixture of local anaesthetics) cream with or without ultrasound exposure, tested on the central forearms of healthy human subjects.[122] EMLA cream placed on an ultrasound-treated site resulted in statistically significant less pain than the placebo cream at each time point. The onset of cutaneous anaesthesia after ultrasound pretreatment was rapid.

Katz and colleagues examined the speed of onset of cutaneous anaesthesia by EMLA cream after brief (approximately 10 s) pretreatment of the underlying skin with low-frequency (55 kHz) ultrasound.[106] Low-frequency ultrasound pretreatment appeared to be safe and effective in producing rapid onset of action by EMLA cream in this model, with results as early as 5 min.

Liposomes

Vyas and colleagues studied liposomally encapsulated diclofenac for sonophoresis-induced systemic delivery.[123] Liposomes containing diclofenac were incorporated into an ointment base for topical application. The systemic availability of drug from liposomes following topical application was evaluated in rats. The effect of sonophoresis on drug release profile was also established *in vitro*. The application of liposomal diclofenac resulted in localisation of the drug at the site of application, with slow systemic availability; the application of ultrasound pulses increased systemic drug levels.

Huang and colleagues used ultrasound to improve the efficiency of liposomal gene transfer.[124] They have developed cationic acoustic liposomes whose composition and structure enables them to reflect ultrasound.

Solid lipid microparticles

El-Kamel and colleagues investigated the effect of permeation enhancers and application of low- and high-frequency ultrasound on transdermal permeation of testosterone after application of testosterone solid lipid microparticles (SLMs).[118] Application of drug-loaded SLMs offered skin protection against the irritation effect produced by testosterone and 1% dodecylamine. Histological characteristics of the skin were affected to various extents by application of enhancers or ultrasound. In general, application of low-frequency ultrasound gave higher testosterone permeation than high-frequency ultrasound. However, safe application of low-frequency ultrasound requires careful selection of exposure parameters.

Microspheres

Supersaxo and colleagues reported macromolecular drug release from biodegradable poly (lactic acid) microspheres.[125] Drug release from porous poly (lactic acid) microspheres showed an initial burst followed by sustained release over several months. When ultrasound was applied to this release system, pulsatile and reversible drug release was observed. The authors speculated that ultrasonic exposure resulted in the enhancement of water permeation within the polymer matrix of the microspheres, inducing drug dissolution into the releasing media.

Matrices

Miyazaki and colleagues used ultrasound to achieve up to a 27-fold increase in the release of 5-fluorouracil from an ethylene and vinyl acetate matrix.[126] Increasing the strength of ultrasound resulted in a proportional increase in the amount of 5-fluorouracil released.

Kost and colleagues described an ultrasound-enhanced polymer-degradation system.[127] During polymer degradation, incorporated drug molecules were released by repeated ultrasonic exposure. As degradation of biodegradable matrix was enhanced by ultrasonic exposure, the rate of drug release also increased. Thus, pulsed drug delivery was achieved by the on/off application of ultrasound.

Increase in the rate of *p*-nitroaniline delivery from a polyanhydride matrix during ultrasonic irradiation was also reported by Kost and colleagues, who noted that the increase in drug delivery was greater than the increase in matrix erosion when ultrasound triggering was active. Thus, it was hypothesised that acoustic cavitation by ultrasonic irradiation was responsible for the modulated delivery of *p*-nitroaniline.[128]

Occlusive dressings

Saliba and colleagues determined the effect of ultrasound on the transcutaneous absorption of dexamethasone (2 g 0.33% cream) applied to the anterior forearm of healthy subjects and

occluded with a dressing.[113] The rate of appearance and the total concentration of dexamethasone in serum were greater in subjects after phonophoresis than after sham ultrasound.

In addition to the above-mentioned dosage forms, the most common way of using drugs in sonophoresis research are as aqueous solutions or drug mixed in coupling gel.[129]

Sonophoresis in conjunction with other enhancement techniques

Sonophoresis and chemical enhancers

Ultrasound is known to act on the skin barrier itself rather than on the inherent mobility of the permeant, and it has been suggested that the effects of sonophoresis may act synergistically with other enhancement methods such as chemical enhancers.[130] The studies described in this section are summarised in Table 3.[131–134]

Matinian and colleagues studied the effect of papain and DMSO phonophoresis. A 1% papain solution together with DMSO enhanced with ultrasound was effective for the treatment of purulent wounds and inflammatory infiltrates.[135] Romanenko & Araviĭskiĭ applied amphotericin B ointment after preliminary treatment with DMSO. Three hours after the application, the maximum content of the antifungal agent in the skin and subcutaneous fatty tissue was higher than after the ointment application that was sonicated but without pretreatment with DMSO. These researchers concluded that both ultrasound and DMSO were enhancers of transcutaneous drug delivery, with DMSO serving as an immediate but short-lived enhancer and ultrasound as a more long-lasting enhancer.[131]

Johnson and colleagues reported that the combination of linoleic acid and ethanol with ultrasound increased corticosterone flux from saturated solutions by up to 13 000 fold relative to the passive flux from phosphate-buffered saline.[132] Similar enhancements were obtained with linoleic acid/ethanol with or without ultrasound for four other model drugs: dexamethasone, estradiol, lidocaine and testosterone. The permeability enhancement for all of these drugs resulting from the addition of linoleic acid to 50% ethanol increased with increasing drug molecular weight.

The effects of low-frequency sonophoresis combined with chemical enhancers such as monoterpenes (L-menthol, L-calvone and D-limonene), laurocapram (azone), glycerol monocaprylate, isopropyl myristate and ethanol on the skin permeation of aminopyrine have been evaluated. The most impressive results were found with the monoterpenes, which have been shown to increase permeant diffusivity in the stratum corneum.[133]

Meidan and colleagues considered the synergy between high-frequency sonophoresis and the chemical enhancers azone and oleic acid on the topical delivery of hydrocortisone.[134] Although ultrasound plus azone resulted in a significant improvement in transport, the use of sonophoresis with oleic acid was less effective.

Mitragotri and colleagues showed that application of 1% sodium lauryl sulfate (SLS) or ultrasound alone for 90 min increased skin permeability to mannitol by about threefold and eightfold, respectively, but in combination induced about a 200-fold increase in skin permeability to mannitol.[105] Specifically, in the absence of surfactants, the threshold ultrasound energy for producing a detectable change in skin impedance was about 14 J/cm^2. Addition of 1% SLS to the solution decreased the threshold to about 18 J/cm^2. Mitragotri and colleagues successfully applied the synergistic effect of ultrasound with SLS in transdermal extraction of analytes *in vitro* and *in vivo*.

Tezel and colleagues reported the synergistic effect of low-frequency ultrasound and surfactants on skin permeability using the model permeant sulforhodamine B, showing that ultrasound enhanced surfactant delivery and dispersion into the skin.[115]

Liu and colleagues investigated the synergistic effect of the chemical enhancers azone and SLS on topical delivery of ciclosporin, reporting that the efficacy of low-frequency ultrasound in enhancing topical delivery could be increased by pretreatment of skin with chemical enhancers.[108] The enhanced skin accumulation of ciclosporin by the combination of low-frequency ultrasound and chemical enhancer could help optimise the targeting of drug without a concomitant increase in systemic side-effects.

Recently, El-Kamel and colleagues studied the effect of sonophoresis and chemical permeation enhancers such as 1% oleic acid and 1% dodecylamine on transdermal delivery of testosterone in an in-vitro study.[118] Application of 1% dodecylamine or 1% oleic acid plus high-frequency ultrasound for 30 min increased permeation rates equally. Hence, it is concluded that synergism of this technique with chemical enhancers leads to better permeation and reduction in threshold energy.[24]

Ultrasound and iontophoresis

Combined application of ultrasound and iontophoresis also has practical applications. The combination of ultrasound and electric current offers a higher enhancement compared with either used individually under similar conditions (Table 4).[136–139] Since ultrasonic pretreatment reduces skin resistivity, a lower voltage is required to deliver a given current during iontophoresis compared with that in controls. This should result in lower power requirements, as well as possibly less skin irritation. Lee and colleagues investigated the effect of ultrasound and iontophoresis on transdermal heparin transport.[136] Ultrasound pretreatment followed by application of iontophoresis enhanced heparin flux by about 56-fold, which was greater than the combined enhancement with ultrasound alone (3-fold) and iontophoresis alone (15-fold).

Fang and colleagues studied the effect of ultrasound and iontophoresis on transdermal transport using a model drug sodium nonivamide acetate (SNA).[137] Pretreatment of skin with low-frequency ultrasound alone did not increase the skin permeation of SNA whereas the combination of iontophoresis and sonophoresis increased transdermal SNA transport more than each method by itself. The enhancement of drug transport across shunt solutes and reduction of the threshold voltage in the presence of an electric field may contribute to this synergistic effect.

Yukio and colleagues tested iontophoresis and sonophoresis alone and in combination on rat back skin using

Table 3 Sonophoresis and chemical enhancers

Drug	Animal/membrane model used	Permeation enhancer used (in italics)	Results	References
Amphotericin B		*DMSO*	Synergistic effect on skin permeation of the drug	Romanenko & Araviĭskiĭ 1991[131]
Dexamethasone, estradiol, lidocaine, testosterone	Human cadaver skin, *in vitro*	1 MHz, 1.4 W/cm^2, C (24 h) *Linoleic acid and ethanol*	Synergistic action increased permeability enhancements for all drugs; increased with increasing molecular weight	Johnson *et al*. 1996[132]
Aminopyrine	–	Monoterpenes (L-menthol, L-calvone, D-limonene)	Synergistic effect showed increased permeant diffusivity in stratum corneum	Ueda *et al*. 1996[133]
Hydrocortisone	Whole rat skin, *in vitro*	1.1 and 3.3 MHz, 0–2.5 W/cm^2, C & P (10 min) *Azone and oleic acid*	Ultrasound plus oleic acid less effective than ultrasound plus azone	Meidan *et al*. 1998[134]
Mannitol	Full-thickness pig skin, *in vitro*	20 kHz, 1.6, 4.5, 6.5, 10 W/cm^2, P (10 min) *Sodium Lauryl Sulfate (SLS)*	Sonophoresis with enhancers caused a synergistic effect over each technique alone	Mitragotri *et al*. 2000[105]
Morphine and caffeine	Hairless mouse skin, *in vitro*	40 kHz, 0.44 W/cm^2, C (4 h) *Propylene glycol, benzalkonium chloride, oleyl alcohol, alpha terpineol*	Comparison of ultrasound plus chemical enhancement indicates a slight superiority of the combination oleyl alcohol/propylene glycol over low-frequency ultrasound	Monti *et al*. 2001[103]
Sulforhodamine B	Full-thickness pig skin, *in vitro*	20 kHz, 7.5 W/cm^2, P (10 min) *Surfactants*	Ultrasound enhanced surfactant delivery and dispersion into the skin	Tezel *et al*. 2002[115]
Ciclosporin A	Rat skin, *in vitro*	20 kHz, 0.4, 0.8 and 1.2 W/cm^2, P (30 min) *Azone and SLS*	Enhanced skin accumulation of drug by combination of ultrasound and SLS help to optimise drug targeting without concomitant increase in side effects	Liu *et al*. 2006[108]
Testosterone	Rat abdomen skin, *in vitro*	1 MHz, 0.5 W/cm^2, C (1 h) and 20 kHz, 2.5, 3.25, 5 W/cm^2, P (30 min) *Oleic acid (OA) and dodeclylamine (DA)*	Higher enhancement in transdermal permeation shown by 1% DA than 1% OA. Application of 1% DA for 30 min after exposure of skin to high- or low-frequency ultrasound had no enhancement effect over application of ultrasound alone	El-Kamel *et al*. 2008[118]

C, continuous mode; P, pulsed mode.

Table 4 Sonophoresis and iontophoresis in combination

Drug	Animal/membrane used	Experimental conditions	Results	References
Heparin	Pig skin, *in vitro*	20 kHz, 7.4 W/cm^2, P (1 h)	Combined treatment resulted in 56-fold increase vs ultrasound alone (3-fold) and iontophoresis alone (15-fold)	Lee *et al.* 2000[136]
Sodium nonivamide acetate	Nude mouse skin	20 kHz, 0.2 W/cm^2, Pretreatment of skin (for 2 h)	Synergistic increase in transdermal drug transport, whereas ultrasound alone did not increase drug permeation	Fang *et al.* 2002[137]
Ascorbic acid	Rat back skin	Sonophoresis and iontophoresis combined	Combined use promoted the absorption of drug	Yukio *et al.* 2006[138]
Vitamin B_{12}	Hairless mice skin, *in vitro*	300 kHz, 5.21 W/cm^2, P (10, 20, 30 min)	Synergism seen, but different mechanism than with ultrasound and iontophoresis treatment alone	Shirouzu *et al.* 2008[139]

P, pulsed mode.

^{14}C-ascorbic acid. They observed that permeation rates were higher in both the epidermis and dermis with the combined treatment combined with either sonophoresis or iontophoresis alone.[138] The combination of iontophoresis and sonophoresis was also administered to the cheek region of male subjects and the metabolic deposition of collagen was examined by measuring the amount of hydroxyproline. From these results it was suggested that the combined use of iontophoresis and sonophoresis promoted the permeation of collagen synthesis.

Shirouzu and colleagues investigated the effect of ultrasound and iontophoresis on skin penetration of vitamin B_{12} as a model drug with a large molecular weight in the stratum corneum of hairless mice *in vitro*.[139] Ultrasound treatment (frequency 300 kHz, intensity 5.21 W/cm^2, pulse mode 5.4% duty cycle) under sonophoresis increased both vitamin B_{12} solubility and diffusivity in the skin according to its energy flux (J/cm^2). The penetration flux of vitamin B_{12} treated with ultrasound of 510 J/cm^2 was 12 times larger than that through intact skin. Using ultrasound and iontophoresis together may have resulted in synergism through a different mechanism than the one responsible for enhancing skin penetration with only ultrasound or iontophoresis, and may be an effective method for skin penetration of large molecules which enter into systemic circulation with great difficulty.

The advantages of this combination include the fact that ultrasound and iontophoresis enhance transdermal transport through different mechanisms, thus making this combination rational. The limitations of this method may include the possibility of requiring a relatively complex device compared with ultrasound or iontophoresis alone.

Ultrasound and electroporation

Transdermal electroporation involves the application of short (1 s), high-voltage (50–500 V) pulses to the skin to cause disorganisation of the stratum corneum lipid structure and thereby enhance drug delivery. Table 5 summarises the studies reviewed in this section.[140]

Kost and colleagues investigated the synergistic effect of therapeutic ultrasound and electroporation on transdermal transport of two molecules, calcein and sulforhodamine.[140] Ultrasound reduced the threshold voltage for electroporation as well as increasing transdermal transport at a given voltage. The enhancement of transdermal transport induced by the combination of ultrasound and electroporation was greater than the sum of enhancement induced by each enhancer alone.

Liu and colleagues recently demonstrated that application of ultrasound or electroporation alone for 6 h did not markedly enhance transdermal delivery of ciclosporin

Table 5 Sonophoresis and electroporation in combination

Drug	Animal/membrane model used	Experimental conditions	Results	References
Calcein and sulforhodamine	Full-thickness human cadaver skin, *in vitro*	1 and 3 MHz, 1.4 W/cm^2, C (1 h)	Enhancement of transdermal drug transport with combination was higher than sum of enhancement induced by each alone	Kost *et al.* 1996[140]
Ciclosporin	Rat skin, *in vitro*	20 kHz, 0.4, 0.8 and 1.2 W/cm^2, P (30 min)	Ultrasound or electroporation alone did not markedly increase transdermal delivery whereas combination increased drug transport to 7 μg/cm^2	Liu *et al.* 2006[108]

C, continuous mode; P, pulsed mode.

(1 and 2.5 μg/cm^2, respectively), whereas simultaneous application of ultrasound and electroporation enhanced transdermal transport to 7 μg/cm^2.[108] Trimodal treatment comprising pretreatment with azone plus ultrasound in combination, followed by electroporation enhanced transdermal ciclosporin transport to 12 μg/cm^2.

Other than this report, there are very few reports of experimental data on combination therapy of ultrasound and electroporation. Also, once synergy has been observed, the practical usefulness and the practicality of such dual technology approaches must be questioned and a dose of realism is perhaps necessary.

Safety issues

The safety aspects of sonophoresis involve the skin barrier properties after turning ultrasound off, and the effect of ultrasound on the living parts of skin and underlying tissues.[87] Numerous reports suggest that application of therapeutic ultrasound (1–3 MHz, 0–2 W/cm^2) does not induce any irreversible change in skin barrier properties.

The World Federation for Ultrasound in Medicine and Biology (www.wfumb.org) has issued several publications relating to the safety of ultrasound bioeffects and non-thermal bioeffects, in an attempt to adopt a policy on safety guidelines.[141,142] Significant efforts have been made to evaluate the safety of low-frequency ultrasound exposure in clinical and laboratory studies.[38,143] As far as the effects of ultrasound on the integrity of skin structure are concerned, a number of histological studies have been performed. At low intensities, no physical damage to skin or the underlying muscle tissues exposed to ultrasound at 20 kHz has been observed.[102] Using optical and electron microscopy, Boucaud and colleagues evaluated structural modifications in human skin after exposure to 20 kHz ultrasound.[144] Human skin samples exposed to intensities lower than 2.5 W/cm^2 showed no modifications *in vitro*, while 5.2 W/cm^2 resulted in epidermal detachment and oedema of the upper dermis. Histological changes such as detachment of the epidermis and dermal necrosis were seen after an exposure to continuous ultrasound at 4 W/cm^2. Further side-effects were observed at higher intensities. Tolerance of low-frequency ultrasound by patients has also been reported in a number of clinical studies.[145,146] Selection of appropriate parameters is crucial in order to apply low-frequency ultrasound safely in a clinical setting. Several parameters, including frequency, intensity, duty cycle, application time, distance of horn and tissue type can influence the results. Further research focusing on safety issues is required to evaluate the limiting ultrasound parameters for safe exposure.

Other applications of sonophoresis

Ocular delivery

Ultrasound has the potential to provide an efficient and minimally invasive method for drug delivery into the eye. Application of 1 s bursts of 20 kHz ultrasound at spatial-average pulse-average intensity of 14 W/cm^2 (spatial-average temporal-average intensity 2 W/cm^2), for enhancement of corneal permeability to glaucoma drugs of different lipophilicity (atenolol, carteolol, timolol and betaxolol), was investigated. The permeability of rabbit cornea increased by 2.6 times for atenolol, 2.8 for carteolol, 1.9 for timolol and 4.4 times for betaxolol (all $P < 0.05$) after 60 min ultrasound exposure *in vitro*. The differences between the treatment and control experiments were significant after 10–30 min ultrasound exposure for all four drugs. In the treatment of corneal infections, the application of 880 kHz ultrasound resulted in up to a 10-fold increase in corneal permeability for sodium fluorescein whilst producing only minor and reversible changes in the corneal structure.[117]

Nail delivery

It was recently reported that ultrasound can also be used for nail delivery of drugs. Torkar and colleagues reported that low-frequency ultrasound enhanced the permeability of the model nail plate to topically applied drugs.[147] Studies to optimise the ultrasound parameters (sonication time, intensity, duty cycle, probe shape, size and distance of horn from the membrane used), which are expected to increase the drug permeation, are underway to understand the mechanisms involved.

Gene therapy

Another future application for ultrasound as a topical enhancer that seems to show promise lies in the field of topical gene therapy.[148] There is considerable interest in facilitating the transfer of genes into diseased tissues and organs. The main aim is to increase the delivery efficiency of exogenous nucleic acid to the intended target. The ideal system would enhance gene expression in the target while having no effect in non-target tissues. Ultrasound might be able to provide this localisation. Ultrasound has been shown to enhance gene transfer into cells *in vitro*[149,150] and *in vivo*.[151] Significantly better transfection is achieved in the presence of cavitation.[152] Enhanced gene transfer is found either when the exposed bubbles are in the vicinity of the genetic material or when genes are encapsulated within or bound to the bubbles. Both strategies have been investigated *in vitro*[153,154] and *in vivo*.[155–157] Ultrasound-enhanced gene therapy is a rapidly evolving field. The exposure levels required to destroy microbubbles lie in the diagnostic range. This is one of the most rapidly expanding fields of ultrasound therapy research; its future utility is of course closely related to the success of gene therapy treatments more widely. A recent themed issue in *Advanced Drug Delivery Reviews* discussed ultrasound in gene and drug delivery in detail in its reviews.[158]

Drug and gene delivery to the brain

According to Raymond and colleagues,[159] low-intensity focused ultrasound with a microbubble contrast agent can be used to transiently disrupt the blood–brain barrier, allowing non-invasive localised delivery of imaging fluorophores and therapeutic/immunotherapeutic agents directly to amyloid plaques in mouse models of Alzheimer's disease. This approach should aid preclinical drug screening and the development of imaging probes. Furthermore, this technique

may be used to deliver a wide variety of small and large molecules to the brain for imaging and therapy in other neurogenerative disorders.

Vaccines

Topical delivery of vaccines such as the tetanus toxoid offers several advantages over needle-based immunisations, including ease of administration. Tezel and colleagues used low-frequency ultrasound (20 kHz, 2.4 W/cm^2) to deliver tetanus toxoid (150 kDa) in mice (Figure 2) and generated a robust immune response.[160] Specifically, low-frequency ultrasound delivered 1.3 μg toxoid into skin, which generated the same immunoglobulin G antibody titres generated by 5 μg subcutaneous injections of tetanus toxoid, sufficient to protect against a lethal dose of tetanus toxin.[161]

Sports medicine

A new direction for ultrasound therapy has been revealed by recent research demonstrating a beneficial effect of ultrasound on injured bone. During fresh fracture repairs, ultrasound reduced healing times by 30–38%.[48] When applied to non-united fractures, it stimulated union in 86% of cases. These benefits were generated using low-intensity (<0.1 W/cm^2) pulsed ultrasound. Though currently developed for intervention in bone injuries, low-intensity pulsed ultrasound has the potential for use on other tissues and conditions more commonly encountered in sports medicine.

Hormone replacement therapy

Kost and colleagues suggested the feasibility of ultrasound as a possible approach to externally affect the release rates of implantable contraceptive delivery systems.[162] Poly (lactide-co-glycolide) microspheres loaded with norethisterone were exposed for 2 h to ultrasound at 3 W/cm^2 (1 MHz, 20% duty cycle) for six consecutive days, resulting in depletion times fourfold shorter than with microspheres that were not exposed to ultrasound. Henzl discussed passive transdermal delivery systems and the possibility of using active transdermal delivery systems including sonophoretic drug delivery for transdermal hormone replacement therapy.[163]

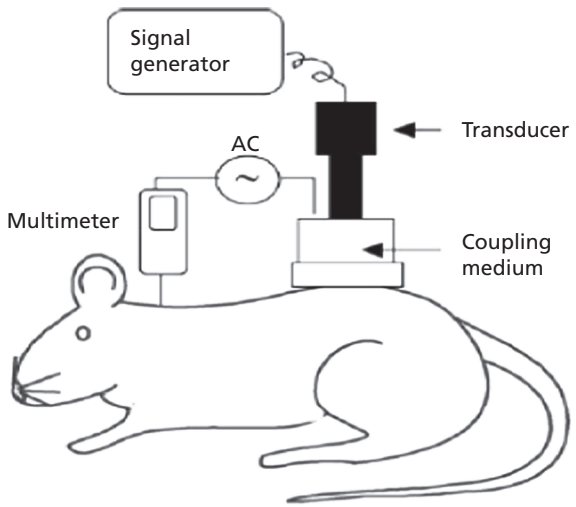

Figure 2 Representation of in-vivo experimental set up in sonophoretic studies[160]

Sonoporation and sonodynamic therapy

Chemical activation of drugs by ultrasound energy for the treatment of cancer is another new field recently termed 'sonodynamic therapy'.[43] Husseini and colleagues demonstrated that cavitation can also aid delivery of drug contained within pluronic micelles.[164] They used doxorubicin inside the hydrophobic core, and showed that the amount of drug released correlated well in subharmonic emissions (70 KHz, 0.28 W/cm^2). Larkin and colleagues showed that application of low-intensity ultrasound to growing tumour enhances intracellular delivery of bleomycin after intraperitoneal or intratumoral administration, thereby potentiating its cytotoxicity.[165] Ultrasound parameters for in-vivo bleomycin delivery were optimised, and an effective antitumour effect was demonstrated in solid tumours of both murine and human cell lines. Cell death after treatment was shown to occur by an apoptotic mechanism. The results achieved in these experiments were equivalent to those achieved using electrochemotherapy.

Sonothrombolysis

Despite a number of successful studies using ultrasound on its own,[46] it was found that more enhancement is achieved when ultrasound exposure is combined with fibrinolytic drugs such as streptokinase, urokinase or tissue plasminogen activator.[166] An interesting application for therapeutic sonography is the thrombolytic effect of ultrasound. A positive effect of ultrasound on clot dissolution was first reported by Trubestein and colleagues.[167] Three different therapeutic options based on ultrasound alone are currently in use: transcutaneous non-invasive ultrasound thrombolysis,[168] catheter-delivered transducer-tipped ultrasound thrombolysis[169] and catheter-delivered ultrasound transducer for thrombolysis.[170,171] All of them use physical properties of ultrasound such as acoustic streaming, shear stress and thermal effects to increase mechanical fragmentation of the thrombus or the enzymatic activity of the applied thrombolytics.

Nanoparticles

New technologies combine the use of nanoparticles with acoustic power for both drug and gene delivery. Ultrasonic drug delivery from micelles usually employs polyether block copolymers, and has been found effective for treating tumours *in vivo*. Ultrasound releases drug from micelles most probably via shear stress and shock waves from collapse of cavitation bubbles. Liquid emulsions and solid nanoparticles are used with ultrasound to deliver genes *in vitro* and *in vivo*. The small packaging allows nanoparticles to penetrate into tumour tissues. Ultrasonic drug and gene delivery from nanocarriers has tremendous potential because of the wide variety of drugs and genes that could be delivered to targeted tissues by fairly non-invasive means.[172]

Cardiovascular therapy

Ultrasound-targeted microbubble destruction is a promising new method that could combine low invasiveness with possibly higher gene transfer efficiency as well as high organ specificity. It is based on the development of second-generation ultrasound contrast agents. These are microbubbles that are stable for several minutes in the human circulation and can pass through the pulmonary capillaries; they can be visualised and destroyed by conventional echocardiography devices. The development of myocardial contrast echocardiography was an essential milestone in this process, as the use of ultrasound-targeted microbubble destruction for local drug and gene delivery is broadly based on tools that were developed for this technique.[173] Ultrasound-targeted microbubble destruction has been shown to increase transfection rates of naked plasmid DNA and viral vectors by several orders of magnitude.[174,175] Ultrasound transducer-tipped catheters are being developed for treatment of cardiovascular diseases.[176]

Commercial sonophoretic systems

Patch-Cap and U-strip

In June 2005, Dermisonics obtained the patent for the ultrasonic Patch-Cap and a flexible patch for transdermal delivery of drugs via ultrasound.[177] This has resulted in the U-strip drug delivery system, which incorporates a transdermal patch in combination with microelectronics and ultrasonic technology.[178] The U-strip Insulin System is the first wearable, programmable and non-invasive drug delivery system that eliminates painful needles and promises improved compliance with the automatic 'set-in and-forget-it' design of the system.[179] The U-strip Insulin Patch is an ultrasonic drug delivery system using an alternating sonic transmission to effect pore dilation and deposit large-molecule drugs into the dermis; it is currently in phase 2 trials.

Sonoderm Technology

Sonoderm Technology is an ultrasound-assisted transdermal transport useful for many drugs, particularly large molecules such as insulin which cannot be administered orally and have to be injected frequently. ImaRx has developed novel ultrasound-enhanced transdermal drug delivery systems.[180] ImaRx is now developing SonoLysis which involves the administration of their MRX-801 microbubbles and ultrasound with or without thrombolytic drug to break up blood clots and restore blood flow to oxygen-deprived tissues. MRX-801 microbubbles are a proprietary formulation of a lipid shell encapsulating an inert biocompatible gas.[181]

Microlysis

The Microlysis developed by Ekos is designed to deliver ultrasound and thrombolytic (clot-dissolving) drug directly into the area of a brain clot.[182] The Microlysis device is a miniature catheter that is inserted into an artery in the brain until it reaches the clot. Drug is infused through the catheter to the tip, where a tiny ultrasound transmitter is located. The ultrasound and drug are designed to be administered simultaneously because it has been shown that ultrasound energy induces a temporary change in the structure of a clot that allows the drug to penetrate more efficiently into the inner reaches of the blockage. Ekos is currently focusing its research and development efforts in the areas of ultrasound-enhanced thrombolysis for treatment of stroke and peripheral vascular occlusion, and gene therapy for prevention of coronary restenosis. Ekos developed the EkoSonic Endovascular System (EkoSonic ES) with rapid pulse modulation for the dissolution of vascular blood clots. This is the only endovascular system that can deliver microsonic energy and thrombolytic drugs simultaneously, providing a safer, faster and more complete way to remove clots by accelerating dissolution. The EkoSonic ES recently received approval by the US Food and Drug Administration.

SonoPrep

Sontra has developed a novel non-invasive and painless skin permeation technique that uses ultrasound to permeate the skin (Figure 3).[183] This device provides a convenient way to enhance permeability through a short (15 s) well-controlled burst of low-frequency (compared with diagnostic imaging) ultrasonic energy to the skin that allows sustained permeability for up to 24 h. The transport properties of the stratum corneum are greatly improved after skin permeation and the technique opens up the potential for non-invasive transdermal diagnostics and the enhanced delivery of drugs through the skin. Initial studies in the area of transdermal drug delivery suggest that ultrasound-mediated skin permeation can enhance transport rates across the skin up to 100-fold for both small and large compounds. Sontra is investigating the delivery of several large proteins and peptides by incorporating the use of the SonoPrep device in combination with transdermal patches to deliver the drug transdermally.[146]

Sontra Medical is developing a vaccine against dengue fever for the US army using the SonoPrep ultrasonic skin permeation device.

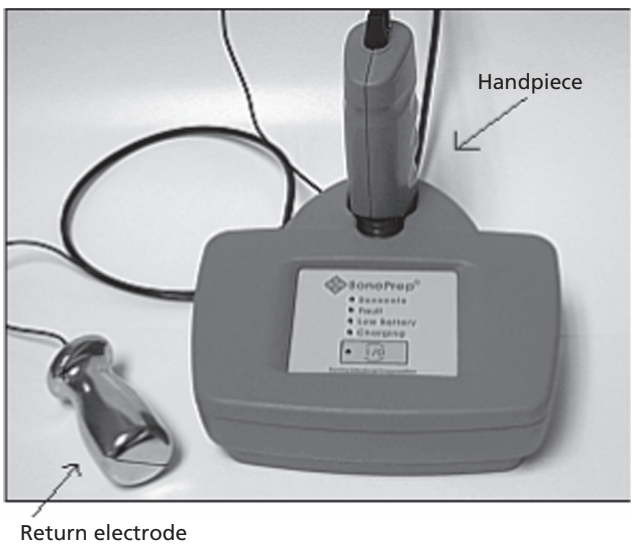

Figure 3 The SonoPrep ultrasonic skin permeation device[183]

Ultra-Sonic Technologies

The main goal of Ultra-Sonic Technologies, LLC is to develop an ultrasonic device for painless transdermal drug delivery based on Dr Ludwig Weimann's patented design.[184] The company also provides custom services for the development of transdermal and topical drug delivery patches with controlled release of the active substance from the device. The company patented an apparatus for performing in-vivo sonoporation of a skin area and transdermal and/or intradermal delivery of a drug solution that comprises a container covered at one end with a porous membrane and containing the drug solution and an ultrasound horn with the tip submerged in the drug solution. The ultrasound horn applies ultrasound radiation to the drug solution.

Thus, sonophoresis has metamorphosed from a crude experimental technique to a highly sophisticated drug delivery technology that is moving closer to commercialisation.

Questions to be resolved before clinical applications

Many concerns have to be addressed before this system can become a clinical reality, such as the appropriate frequencies, pressure amplitudes, formulations, amount of coupling medium, distance of ultrasound horn from the skin, and so on. More research needs to be conducted in order to identify the role of the various parameters that influence phonophoresis so that the process can be optimised. Are the structural alterations generated by ultrasound bioreversible? How often and for what duration should ultrasound be used to maximise local absorption of drugs? Which topical drugs can most effectively be used for phonophoresis? What kind of in-vivo studies are needed to investigate tolerance and transdermal transport in humans? What considerations are necessary in the development of a convenient and cost-effective ultrasound device?

Scope of future research

Ultrasound-mediated drug therapy has immense future and scope for further research. Unfortunately to date most of this treatment has been conducted on a rather subjective and non-quantitative basis[185] and is plagued by lack of use of proper controls, incomplete accounts of dosimetry and vagueness in designing experimental protocols.[8,186] The conflicting data have resulted from the fact that different research groups have used different ultrasonic parameters (i.e. frequency, intensity, duration, mode), different skin membranes and different vehicles. In addition, the presence and absence of cooling systems, processing of membranes used, distance between skin and transducer, size of transducer, quantity and type of coupling medium used, and end point evaluation techniques all affect the sonophoretic skin permeation rates.[129,187]

Phonophoretic research often suffers from poor calibration in terms of the amount of ultrasound energy emitted.[188,189] The problem is that as an ultrasound propagates away from its source, the beam area begins to expand after a certain critical distance. Mathematically, this is dependent on the ultrasonic wavelength, transducer radius and effects associated with constructive and destructive wave interference.[190] Ultrasound can reflect back on itself at a tissue–bone interface *in vivo* or at a vessel wall–solution interface *in vitro* to produce a standing wave. However, to date no research has been published on the effect of ultrasound standing waves on drug migration, either *in vivo* or *in vitro*.[11]

An important area that needs attention is understanding of the biophysical mechanisms involved in ultrasound–tissue interaction, which are not yet fully understood. This lack of understanding is because several phenomena may occur simultaneously in skin upon ultrasound exposure, such as cavitation, thermal effects, convective transport and mechanical effects. But, if one can identify the dominant phenomena responsible for sonophoresis, a better selection of ultrasound parameters can be made to selectively enhance the favourable phenomena and thereby enhance the efficacy of this system.[191]

Another factor that must not be neglected is the effect of ultrasound on drug stability.[129] Degradation of drugs in the presence of ultrasound has been studied and reported in only a few cases, for example, oligonucleotides, insulin, fentanyl and caffeine.[2,11,101,102,192] No degradation was reported. However, this important aspect should be studied as a part of preformulation studies for any drug to be used through sonophoretic delivery. Establishment of long-term safety issues, broadening the range of drugs that can be delivered through this system, as well as reducing the cost of delivery are issues that still needs to be addressed.[15,193]

Conclusions

It can be concluded beyond doubt that ultrasound can markedly increase percutaneous absorption. Understanding of the mechanisms by which biological effects are produced is still insufficiently understood, and more recent research on this is indicated if the therapeutic potential of ultrasound is to be fully realised. There is need for greater collaboration between medical physicists and pharmaceutical scientists so that knowledge of the biophysical interactions of ultrasound can be linked to established technology. Synergistic effects of various technologies like chemical enhancers, iontophoresis and electroporation with sonophoresis have been reported but detailed in-vivo investigations are also required to fully assess simultaneous application of ultrasound and other techniques. Further studies should also address microscopic details of the mechanisms by which such synergistic combinations increase skin permeability. A few prototype devices have been developed for the preliminary testing and the results have demonstrated their effectiveness for transdermal drug delivery. Further work is currently being pursued using design simulation methods, microfabrication techniques and biocompatible polymers to develop new sonophoresis microdevices. Sonophoresis is an attractive and competitive technology for drug delivery, but will have to overcome much tougher obstacles than its passive counterparts before it can make a lasting impact in the years to come.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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