

Expert Opinion

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Low-frequency sonophoresis: application to the transdermal delivery of macromolecules and hydrophilic drugs

Baris E Polat, Daniel Blankschtein[†] & Robert Langer

Massachusetts Institute of Technology, Department of Chemical Engineering, Cambridge, MA, USA

Importance of the field: Transdermal delivery of macromolecules provides an attractive alternative route of drug administration when compared to oral delivery and hypodermic injection because of its ability to bypass the harsh gastrointestinal tract and deliver therapeutics non-invasively. However, the barrier properties of the skin only allow small, hydrophobic permeants to traverse the skin passively, greatly limiting the number of molecules that can be delivered via this route. The use of low-frequency ultrasound for the transdermal delivery of drugs, referred to as low-frequency sonophoresis (LFS), has been shown to increase skin permeability to a wide range of therapeutic compounds, including both hydrophilic molecules and macromolecules. Recent research has demonstrated the feasibility of delivering proteins, hormones, vaccines, liposomes and other nanoparticles through LFS-treated skin. *In vivo* studies have also established that LFS can act as a physical immunization adjuvant. LFS technology is already clinically available for use with topical anesthetics, with other technologies currently under investigation.

Areas covered in this review: This review provides an overview of mechanisms associated with LFS-mediated transdermal delivery, followed by an in-depth discussion of the current applications of LFS technology for the delivery of hydrophilic drugs and macromolecules, including its use in clinical applications.

What the reader will gain: The reader will gain an insight into the field of LFS-mediated transdermal drug delivery, including how the use of this technology can improve on more traditional drug delivery methods.

Take home message: Ultrasound technology has the potential to impact many more transdermal delivery platforms in the future due to its unique ability to enhance skin permeability in a controlled manner.

Keywords: cavitation, drug delivery, macromolecules, proteins, sonophoresis, surfactants, ultrasound, vaccination

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1. Introduction

The delivery of macromolecules or hydrophilic drugs has long been a desired goal in transdermal drug delivery. This is due to the fact that the skin provides an attractive alternative route of drug administration compared to oral delivery and hypodermic injections. With respect to oral delivery, the advantages include: i) minimization of first-pass metabolic effects, ii) bypassing of the harsh gastrointestinal tract that can cause degradation or denaturing of macromolecules and iii) decreased side effects from dyspeptic drugs [1,2]. Furthermore, the advantages of transdermal delivery over hypodermic injections include: i) non-invasive delivery, ii) decreased pain

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Article highlights.

- The general field of transdermal drug delivery, its benefits over other delivery methods, and the structure and barrier properties of the skin are introduced.
- Background into the mechanisms of enhancement by low-frequency sonophoresis (LFS) and observed phenomena in skin treated by LFS are presented.
- Theoretical models and experimental methods utilized in the delivery of hydrophilic permeants with LFS are discussed.
- Research involving LFS-mediated delivery of high-molecular weight molecules and nanoparticles is analyzed.
- Current use of LFS technology in patients and applications of the LFS technology that are under investigation for regulatory approval are discussed.
- Issues related to the safety of LFS treatment are examined.
- Exciting current and future applications are examined, including transcutaneous immunization and a closed-loop system for glycemic control, including other forward looking statements.

This box summarizes key points contained in the article.

and increased patient compliance, iii) decreased generation of dangerous medical sharps and iv) less risk of needle contamination, disease transmission and needle misuse [1,2]. Transdermal delivery also allows for sustained release profiles for systemic or localized drug delivery, which is not always possible with oral or injectable formulations. However, the innate structure of the skin provides a very robust barrier to drug delivery. In fact, only very small and hydrophobic molecules, such as clonidine, estradiol, fentanyl, nicotine, nitroglycerine, scopolamine and testosterone, have been successfully administered at therapeutic levels through intact skin [1,2]. This has led to the so-called '500 Dalton Rule', which states that for a drug to be deliverable through native skin, it must have a molecular mass of < 500 Daltons and, in general, be hydrophobic [3]. The reason for these limitations is that the primary barrier for transdermal transport is the outermost layer of the epidermis, the stratum corneum, which is typically only 10 – 20 μm in thickness (Figure 1). The stratum corneum is a non-viable cell layer that is comprised of highly crosslinked keratinocytes (or corneocytes), embedded in a continuous matrix of skin lipids (Figure 2).

To deliver high-molecular mass or hydrophilic drugs, one can generally use one of two approaches. First, if the drug being administered is sufficiently small, chemical modification can be utilized to increase its lipophilicity, without directly affecting the structure of the skin [4]. Furthermore, one can also increase the driving force for permeation using various strategies, including supersaturation of the delivery vehicle, increasing the vehicle-to-skin partition coefficient or by electroosmotic flow induced by iontophoresis, without directly affecting the skin structure [5]. Conversely, one can

use a chemical or physical enhancer to decrease the barrier properties of the skin without modifying the drug itself. Chemical enhancers include molecules such as surfactants or oils, and operate by denaturing keratinocytes, disordering/fluidizing lipid bilayers or creating segregated phases in the stratum corneum [1,2]. There are many types of physical enhancers for transdermal delivery, such as iontophoresis (application of a continuous, low-voltage electric field) [6], electroporation (application of pulsed, high-voltage electricity) [6], thermal or laser ablation [7], liquid or powder jet injectors, [8] or the use of high- or low-frequency ultrasound (sonophoresis). Low-frequency sonophoresis (LFS), in particular, offers advantages over other transdermal delivery methods. Specifically, with LFS, the extent of skin perturbation, and the resulting skin permeability enhancement, can be controlled by varying the application time and other ultrasound parameters [9-13]. Therefore, systemic, regional and local deliveries are possible with LFS. The size of aqueous skin pores created by LFS can also be controlled by varying the frequency and the intensity of the ultrasound utilized [11,14]. Moreover, LFS allows for pretreatment of the skin, prior to application of a drug-containing patch, so that the actual LFS treatment is only on the order of seconds and the device does not need to be worn constantly [15-18]. Finally, LFS has shown promise in delivering both macromolecules and hydrophilic permeants transdermally [19,20], as well as in non-invasively monitoring blood analytes, such as blood glucose [21,22]. In this review, the mechanisms and phenomena associated with LFS are highlighted, followed by a more rigorous analysis of both theoretical and experimental studies on the delivery of large and hydrophilic permeants with LFS. Commentary is also provided on the current clinical status of LFS, including a critical discussion of what the future holds for LFS-assisted transdermal drug delivery.

2. Background

Although the use of therapeutic (0.7 – 3 MHz) and high-frequency (> 3 MHz) ultrasound for medical applications, including transdermal drug delivery, goes back many decades, the use of low-frequency ultrasound (20 – 100 kHz) for transdermal delivery is still in its relative infancy, spanning only the last 2 decades. Initial studies utilizing LFS were reported in the early 1990s by Tachibana and Tachibana, who utilized 105 and 48 kHz ultrasound to transdermally deliver insulin and lidocaine to rabbits and hairless mice [23-25]. Shortly thereafter, Mitragotri *et al.* demonstrated that LFS could be utilized to deliver even higher molecular mass proteins, such as IFN- γ (~ 17,000 Da) and erythropoietin (~ 48,000 Da) [19]. Additional work showed that LFS is up to three orders of magnitude more effective in inducing transdermal transport than therapeutic ultrasound [26]. Therefore, this review focuses only on the mechanisms and applications of low-frequency ultrasound-mediated transdermal drug delivery.

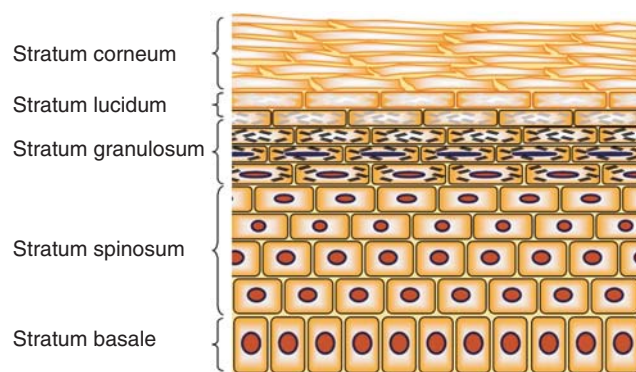


Figure 1. Cross-sectional schematic of the stratified layers of the epidermis. The stratum corneum provides the skin with the majority of its barrier properties, being comprised of anucleate corneocytes embedded in a lipid matrix. Legend: corneocytes (orange rectangle), keratinocytes with a nucleus (blue circle), keratinocytes containing granules (blue circle with granules) and lipids (yellow rectangle).

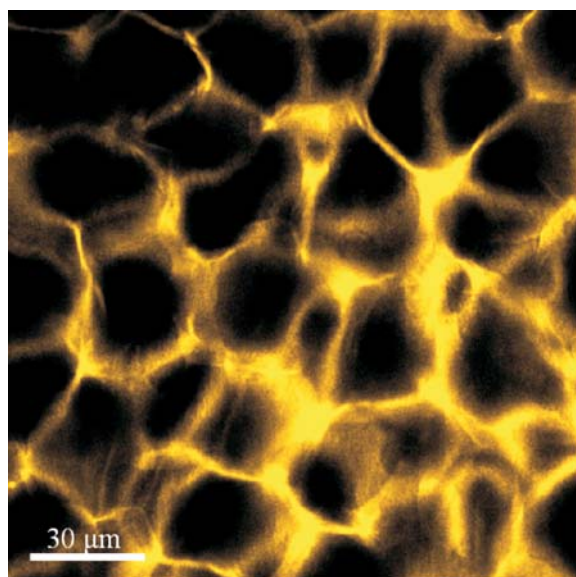


Figure 2. Two-photon microscopy image of the honeycomb structure of the stratum corneum. The lipid bilayers are stained with the fluorescent dye rhodamine B-hexyl ester. The dark, approximately hexagonal, regions are the highly cross-linked corneocytes, where the dye cannot penetrate deeply.

2.1 Mechanisms

After it was established that LFS is significantly more effective at increasing skin permeability than ultrasound at therapeutic frequencies, the next major goal in the field was to achieve a better mechanistic understanding of skin perturbation by LFS. Note that the mechanisms of LFS depend on the treatment protocol utilized. Two protocols have been

reported in the literature: i) the simultaneous protocol, with a drug included in the LFS coupling medium and ii) the pretreatment protocol, which involves the application of LFS followed by the passive delivery of a drug to the skin in a patch or other formulation. The pretreatment protocol tends to be used more often than the simultaneous protocol in more recent studies involving LFS-mediated delivery of macromolecules [27-32]. This is because the simultaneous protocol requires that the ultrasound device be constantly applied to the skin during treatment, which can cause degradation of the drug compound in the coupling medium, thus, providing an additional limitation in comparison to the pretreatment protocol [33,34]. However, some recent studies have also used the simultaneous protocol with a miniaturized LFS device, utilizing a degassed coupling medium in order to minimize the effects of compound degradation [35-41].

The first mechanistic investigations of LFS involved the use of acoustic spectroscopy to gauge the importance of cavitation as an enhancing mechanism. In these experiments, Tang *et al.* and Tezel *et al.* showed conclusively that inertial acoustic cavitation is the primary mechanism of LFS-induced skin permeability enhancement [42,43]. Furthermore, by selectively suppressing cavitation in the bulk coupling medium and in the entire system, Tang *et al.* were able to demonstrate that only cavitation above the skin, in the aqueous coupling medium, is responsible for skin permeability enhancement by LFS [42]. Additional studies followed which established that cavitation in the vicinity of the skin surface, likely as transient cavitation microjets directed at the skin surface, is the most likely contributor to skin permeability enhancement [44]. For an in-depth discussion of the physics of ultrasound and cavitation, the reader is referred to the review by Leighton [45]. Although cavitation within the skin itself can play a role at therapeutic ultrasound frequencies (> 0.7 MHz) [46], it does not play a role in skin permeability enhancement with LFS [42]. Unfortunately, these mechanisms are sometimes confused, leading to incorrect citations. For example, several recent reviews and papers have referenced sonophoresis studies utilizing therapeutic frequencies, or incorrectly cited LFS studies, to state that only cavitation within the skin plays an important role in LFS-mediated transdermal drug delivery [47-49].

With the simultaneous protocol, in addition to the cavitation effects discussed above, convective processes can also play a role. For example, Tang *et al.* have shown that convection can cause increased drug transport in heat-stripped skin, although it does not play a role in thicker skin models such as split-thickness skin (700 μm) and full-thickness skin models [20]. Furthermore, Tachibana and Tachibana have shown that convection can play a role in the transdermal delivery of lidocaine with LFS [25]. However, other studies have concluded that convection does not play a role with LFS [10]. Therefore, the role of convection as an enhancing mechanism in LFS, especially in comparison to cavitation, is still not completely understood.

2.2 Localized transport

A shift in the mechanistic understanding of LFS occurred when it was observed that skin permeability enhancement occurs primarily in discrete regions of the skin, [50,51] and not uniformly across the entire skin surface. These highly-perturbed regions (Figure 3), referred to as localized-transport regions (LTRs), have been shown to be up to three orders of magnitude more permeable than untreated skin [14,52,53]. In addition, when a chemical enhancer, such as the surfactant sodium lauryl sulfate (SLS), is included in the LFS coupling medium, it has been shown that even the less-perturbed non-LTRs possess increased transport properties with respect to untreated skin, although to a considerably smaller extent than the highly-perturbed LTRs [14,52]. Recently, it has been shown that the mechanisms of enhancement within LTRs and non-LTRs are different [14]. By studying the average aqueous pore radii (see Section 3.1) within each skin region at multiple LFS frequencies (20, 40 and 60 kHz), in the presence of SLS, it was shown that pore radii within LTRs are strongly frequency-dependent, while pore radii within non-LTRs exhibit no frequency dependence. This suggests that a frequency-dependent process, such as the collapse of transient cavitation microjets at the skin surface, is responsible for the observed enhancement within the LTRs, while the frequency-independent process of SLS acting on the skin is the main mechanism of enhancement within the non-LTRs [14].

2.3 Synergism between LFS and surfactants

It is well known that using multiple skin penetration enhancers, both chemical and physical, can lead to synergistic skin permeability enhancement. For example, chemical enhancers are known to enhance skin permeability through their interaction with lipid bilayers or via the denaturation of corneocytes [2]. However, many chemical enhancers are limited in their ability to penetrate into the skin and are, therefore, limited in inducing more substantial permeability enhancement due to the natural barrier properties of the stratum corneum. Therefore, if a second enhancer is used that can not only increase the permeability of the skin itself, but also increase the penetration of the first enhancer, a much larger effect is often observed in the combined case compared to either enhancer alone. This mechanism has been shown to be particularly significant in the case of the synergism observed between LFS and surfactants. For example, Mitrangotri has shown that the synergism between LFS and SLS occurs because of increased SLS penetration and dispersion in the skin as a result of the LFS application [54]. In a subsequent study, Tezel *et al.* investigated the synergism between LFS and a group of fourteen separate surfactants [54,55]. Their findings showed that if skin is treated with a given surfactant for a sufficiently long time, the skin permeability enhancement induced by the surfactant alone will approach the enhancement induced by the combination of the surfactant and LFS. This suggests that LFS-induced penetration of surfactant

into the skin plays a central role in the observed synergism between chemical enhancers and LFS [55]. Other mechanisms have also been studied to elucidate the synergism between LFS and surfactants, such as shifts in the pH profile of the skin as a result of the LFS/SLS treatment [56]. However, in spite of these observations, to date, there has been no physical mechanism proposed that demonstrates how LFS causes increased penetration of surfactants into the skin. Therefore, the synergism between LFS and surfactants is still not fully understood and represents a fertile area of ongoing research.

The synergism between LFS and surfactants also has implications on the clinical viability of ultrasound-mediated transdermal drug delivery. For example, it has been demonstrated that the treatment time and energy density required to induce significant skin permeability enhancement are decreased by nearly an order of magnitude when SLS is combined with LFS [54,55]. This allows patients to be treated for much shorter periods of time and with less intense ultrasound protocols, which is extremely beneficial for the practical use of LFS. Moreover, a recent study has shown that the combined LFS/SLS treatment of skin may, in fact, be less perturbing to the skin than that using LFS alone [57]. Although this finding is not intuitive, because it would be expected that the combination of a physical and a chemical enhancer should induce more skin perturbation than a physical enhancer alone, it reflects the times required to reach similar extents of skin permeability using each treatment [57]. Specifically, because the LFS treatment alone takes nearly an order of magnitude more time to reach similar extents of skin permeability than the LFS/SLS treatment, skin samples are subjected to a much harsher treatment when only LFS is utilized. Furthermore, it was shown that skin samples treated by LFS/SLS yielded much more predictable and reproducible skin permeability values than those treated with LFS alone, a finding which is clearly more desirable in a clinical setting [57]. For these reasons, the combination of LFS and a chemical enhancer is nearly always utilized in LFS treatment protocols, particularly in clinical settings or when delivering large or hydrophilic permeants.

3. Delivery of hydrophilic permeants with LFS

3.1 The aqueous porous pathway model

Although traditional transdermal transport models have typically utilized molecular mass and octanol-water partition coefficients ($\log K_{o/w}$) to describe solute transport through the skin, including transport of hydrophilic permeants [58,59], an improved mechanistic understanding of hydrophilic permeant transport through LFS-treated skin was made possible by the development of the aqueous porous pathway model (APPM) [20]. By assuming that hydrophilic permeants traverse the stratum corneum along the same pathways as the current carrying ions, the APPM derives a quantitative relationship between the skin permeability of hydrophilic permeants, P , and the skin electrical resistivity, R . Specifically, by utilizing



Figure 3. LTRs on the surface of low-frequency ultrasound and ethanol treated skin, as visualized with allura red staining.

LTR: Localized transport region.

relations from hindered-transport theory for the diffusion of permeants through confined pores [60], the APPM demonstrates that a linear regression of $\log P$ versus $\log R$ should yield a slope of -1 [20]. Moreover, by substituting the values of known parameters associated with the bulk solution, the skin membrane and the diffusing permeant (hydrodynamic radius), one can calculate important structural parameters of the skin utilizing the APPM, including the average skin aqueous pore radius and the skin porosity:tortuosity ratio. The APPM has been applied to a broad class of hydrophilic permeants (Table 1), for both small molecules and macromolecules, diffusing through LFS-treated skin [12,14,20,32,52,61-63]. Although the APPM assumes a single average pore radius, it can be extended to include: i) a distribution of aqueous pore radii, [32] and ii) lipophilic pathways [64]. There is strong evidence that there is a distribution of skin pore radii, as reflected by the fact that the size of the permeant used in the context of the APPM affects the average pore radius calculated [32,61]. In other words, larger permeants can only travel through aqueous pores that are larger than their hydrodynamic radius. Therefore, macromolecules can only sample the upper tail of the aqueous pore radius distribution, while smaller permeants have access to a wider range of pore radii. In addition to the apparent pore radius depending on the size of the permeant used, the ultrasound amplitude also plays an important role. An examination of Table 1 shows that most studies conducted in the range 7.2 – 7.5 W/cm² at 20 kHz calculated the average skin aqueous pore radius to be close to 100 Å. On the other

hand, studies which utilized 1 – 1.6 W/cm² at 20 kHz generally calculated average skin aqueous pore radii which are smaller than 35 Å (comparable to the radius of native skin pores). It is also worth noting that at lower LFS intensities (1.08 W/cm²), no frequency dependence of the pore radii was observed [32]. On the other hand, at higher LFS intensities (7.5 W/cm²), pore radii were affected significantly by the applied ultrasound frequency [14]. The observed intensity and frequency dependence of pore radii are both likely due to the formation of larger LTRs at higher ultrasound intensities. Indeed, it is known that large aqueous pores are formed almost exclusively within LTRs due to cavitation processes [14,52,64], and that LTR area is inversely proportional to skin resistivity (skin samples with lower skin resistivities generally possess larger LTRs) [14,52]. Therefore, given that the $\log R$ values in Table 1 are lower for the samples treated at higher LFS intensities (7.2 – 7.5 W/cm²), the larger pores and the observed frequency dependence of pore radii in these skin samples are due to an increase in LTR area and to a higher level of skin perturbation in these samples, with respect to samples treated at LFS intensities of 1 – 1.6 W/cm².

The APPM has been very useful to extract information about how various LFS treatment regimens, both in the presence and absence of chemical enhancers, affects the structural state of the skin. For example, a recent mechanistic investigation studied pore radii within LTRs and non-LTRs of LFS-treated skin [14]. By showing that the pore radii within LTRs correlate strongly with the applied LFS frequency, while the pore radii within non-LTRs are frequency-independent, useful conclusions about the enhancement mechanisms operating within each skin region were drawn (see Section 2.2) [14]. In addition, utilizing the APPM, Seto *et al.* have recently shown that the response of different skin models to the LFS/SLS treatment is not necessarily the same. Specifically, it was demonstrated that, although full-thickness and split-thickness pig skin models respond in an equivalent manner to the LFS/SLS treatment, human skin models of varying thickness do not respond in an identical fashion [63]. In general, the APPM provides a useful tool for understanding and analyzing the transport of hydrophilic permeants across the skin, as well as for analyzing the structural state of the skin following LFS treatment.

3.2 LFS-mediated transdermal transport of hydrophilic permeants

The ability of LFS to increase skin permeability to hydrophilic permeants has been well established through research done with the APPM. Simple sugars, such as sucrose and raffinose (Table 1), having $\log K_{o/w}$ < -3 [65] and even larger water soluble fibers, such as inulin, have been shown to penetrate through skin in appreciable amounts following LFS treatment [20,32,57,61,63]. In addition to allowing the delivery of hydrophilic permeants, LFS skin treatment has also been shown to allow the

Table 1. Hydrophilic molecules that have been investigated in the context of the APPM for LFS-treated skin.

Molecule	MM (Da)	Ultrasound parameters				Co-enhancer	Skin model*	APPM results			Ref.
		Freq. (kHz)	Amplitude (W/cm ²)	Skin-to-horn distance (mm)	log R range	Duty cycle		Slope	log C*	Pore radius (Å) [§]	
Mannitol	182	19	1.08	3	0.3 to 1.7	100%	1% SLS	-	-2.92	28 ± 10	[12]
Mannitol	182	58	1.08	3	0.2 to 1.6	100%	1% SLS	-0.85	-2.93	23 ± 13	[32]
Mannitol	182	58	1.08	3	0.1 to 1.9	100%	1% SLS	-	-2.91	29 ± 9	[12]
Mannitol	182	76	1.08	3	0.6 to 1.9	100%	1% SLS	-	-2.92	28 ± 12	[12]
Mannitol	182	93	1.08	3	0.6 to 1.9	100%	1% SLS	-	-2.94	26 ± 8	[12]
Mannitol	182	20	1.6	8	-1.0 to 0.4	10%	None	-0.95	-2.50	> 92	[20]
Mannitol	182	20	1.6	8	-0.4 to 1.6	10%	None	-0.87	-2.98	22 ± 20	[20]
Mannitol	182	20	7.2	3	0.6 to 0.8	50%	1% SLS	-	-	41 [¶]	[61]
Mannitol	182	20	7.2	3	-1.1 to -0.9	50%	1% SLS	-	-	57 [¶]	[61]
Sucrose	342	20	1.6	8	-1.0 to 0.1	10%	None	-0.88	2.61	> 125	[20]
Sucrose	342	20	1.6	8	-0.2 to 1.7	10%	None	-1.05	-3.21	23 ± 10	[20]
Sucrose	342	20	7.5	3	0.0 to 1.6	50%	1% SLS	-1.08	-3.05	39	[63]
Sucrose	342	20	7.5	3	-0.4 to 1.6	50%	1% SLS	-1.04	-2.91	> 120	[46]
Sucrose	342	20	7.5	3	-0.2 to 1.6	50%	1% SLS	-1.09	-2.69	> 120	[46]
Sucrose	342	20	7.5	3	-0.3 to 1.4	50%	1% SLS	-1.07	-2.95	113	[63]
Sucrose	342	20	7.5	3	-0.2 to 1.4	50%	1% SLS	-1.07	-2.89	113	[63]
Sucrose	342	20	7.5	3	-0.3 to 1.5	50%	1% SLS	-1.08	-2.96	> 120	[57]
Sucrose	342	20	7.5	3	-0.1 to 1.3	50%	1% SLS	-1.07	-2.90	> 120	[57]
Sucrose	342	20	7.5	3	0.0 to 1.3	50%	None	-0.98	-2.96	> 120	[57]
Sucrose	342	20	7.5	3	0.0 to 1.3	50%	None	-1.02	-2.68	> 120	[57]
Raffinose	504	20	7.2	3	0.6 to 0.8	50%	1% SLS	-	-	88 [¶]	[61]
Raffinose	504	20	7.2	3	-1.1 to -0.9	50%	1% SLS	-	-	98 [¶]	[61]
Calcein	623	20	7.5	3	-0.2 to -0.8	50%	1% SLS	-0.92	-3.14	105	[14]
Calcein	623	40	7.5	3	-0.2 to -0.8	50%	1% SLS	-1.02	-3.19	73.9	[14]
Calcein	623	60	7.5	3	-0.2 to -0.9	50%	1% SLS	-1.08	-3.26	50.7	[14]
LHRH [#]	1311	58	1.08	3	0.0 to 1.7	100%	1% SLS	-0.74	-3.42	28 ± 4	[32]
Inulin	5000	58	1.08	3	0.1 to 1.8	100%	1% SLS	-0.65	-3.86	34 ± 4	[32]
Inulin	5000	20	7.2	3	0.6 to 0.8	50%	1% SLS	-	-	111 [¶]	[61]
Inulin	5000	20	7.2	3	-1.1 to -0.9	50%	1% SLS	-	-	95 [¶]	[61]
Dextran	70,000	58	1.08	3	0.2 to 1.6	100%	1% SLS	-0.44	-4.40	58 ± 3	[32]

*All skin models are *in vitro*. Legend: p: Pig; h: Human; FTS: Full-thickness skin; HSS: Heat-stripped skin; 700: 700-µm dermatomed skin; and 250: 250-µm dermatomed skin.[†]log C is equivalent to the Y-intercept in a plot of log P vs log R, where the slope is required to be equal to the APPM theoretical value of -1.[§]Publications after 2008 utilized an updated form of the hindrance-factor expression in pore radius calculations.[¶]Pore radii were calculated by comparing permeant permeability to urea permeability rather than to ion permeability (R).[#]LHRH: Luteinizing-hormone-releasing hormone.

APPM: Aqueous porous pathway model; LFS: Low-frequency sonophoresis; MM: Molecular mass; SLS: Sodium lauryl sulfate.

extraction of hydrophilic analytes [21,22,38,66], such as blood glucose ($\log K_{o/w} = -3.24$ [65]). Strict glycemic control is critical for proper care of diabetic patients. By increasing the permeability of the skin using LFS, followed by the application of a transdermal glucose sensor, it has been shown that blood glucose levels can be monitored continuously for up to 24 h [15,67]. The delivery and extraction of hydrophilic permeants in clinical use are discussed further in Section 5.

4. Delivery of macromolecules utilizing LFS

The delivery of proteins, biopolymers, nanoparticles and other high-molecular mass drugs or particles has received considerable attention during the past few years. However, this application of LFS is not just a recent trend, but dates back to some of the earliest studies with LFS [19,24]. The benefit of transdermal protein delivery is that it bypasses the harsh environment of the gastrointestinal tract, which can induce protein denaturation and loss of therapeutic activity. Moreover, compared to injections, it allows for controlled release profiles (not just a bolus) and displays possible compliance and safety benefits due to the elimination of needles from the delivery process [1,2]. Therefore, it is not surprising that when LFS was established as a feasible means of delivering high-molecular mass proteins, some of the first molecules that were tested included proteins, such as insulin and IFN- γ [19,24]. However, delivery of high-molecular mass molecules by LFS is not only limited to proteins, but includes high-molecular mass drugs, hormones, fibers, biopolymers, oligonucleotides, liposomes, nanoparticles and even vaccines (Table 2).

The most widely studied protein, with respect to transdermal delivery by LFS, is insulin (Table 2), because of its implications in the treatment of diabetes. After Tachibana showed the feasibility of transdermal insulin delivery with LFS [24], Mitragotri *et al.* conducted the first comprehensive study comparing LFS-mediated insulin delivery with subcutaneous injection [19]. These authors showed that, above a threshold ultrasound intensity and treatment time, LFS-mediated insulin delivery was as effective at lowering blood glucose levels as subcutaneous injection, including lowering blood glucose levels of diabetic rats to normal levels [19]. More recently, there has been extensive research on insulin delivery using a light, portable cymbal array device, which operates at 20 kHz and intensities between 50 and 100 mW/cm² [36,37,39,40]. The novelty of the cymbal array is its compact size, being only 3 mm in thickness and varying in surface dimensions between 3 cm \times 3 cm and 6 cm \times 6 cm, which allows the device to be worn during treatment. This could potentially lead to a closed-loop system, where one wearable device could house both a glucose sensor and the ultrasound transducer, allowing the delivery of insulin on demand in response to glucose readings. The feasibility of both insulin delivery [36,37,39,40] and glucose sensing [38] using the cymbal array device has been tested and validated.

4.1 Transdermal vaccination

In addition to insulin, another research area that is gaining increased attention is transdermal vaccination. Transdermal vaccination would provide many advantages over current hypodermic injection methods of vaccination because it would completely eliminate safety concerns involving the use of needles, especially with regard to misuse and improper re-use in lower-income areas and nations. Furthermore, using a patch vaccine formulation, it may be possible to expose the body to a lower concentration of antigens for a longer period of time, which could provide a similar, or stronger, immune response by targeting the Langerhans cells within the skin, while having the added safety benefit associated with lower concentrations of antigen encountered by the immune system [68,69]. The first study of LFS-mediated vaccine delivery was conducted by Tezel *et al.*, and involved the delivery of tetanus toxoid (TT) into an *in vivo* mouse model following pretreatment with 20 kHz LFS and 1% SLS [31]. The study found that TT IgG titers increased with increasing LFS energy density (LFS energy density = LFS amplitude \times LFS treatment time) and that the titers obtained with 1.3 μ g of TT delivered by LFS were similar to those obtained by a subcutaneous injection of 10 μ g of TT (note that a subcutaneous injection of 5 μ g of TT is sufficient for immunity to tetanus toxin). Therefore, the authors concluded that LFS not only elicits an enhanced immune response because of the increased delivery of TT to the Langerhans cells, but also through LFS-induced activation of Langerhans cells. In fact, LFS alone, in the absence of antigen, was found to induce activation of Langerhans cells in these studies [31]. Other studies have also shown the ability of high-amplitude LFS (5 – 7 W/cm²) to induce an increased immune response, when compared to low-amplitude LFS (0.15 W/cm²) and negative controls [70]. These findings demonstrate the significant potential of LFS to act as a physical adjuvant and motivate further investigation of LFS-mediated transdermal immunization. A more recent study investigated the level of TT antibody titers in mice treated with LFS, while varying SLS concentrations and ultrasound parameters [28]. This study found that 0.5% SLS provided an immune response superior to that of 1% SLS, despite causing less skin perturbation. Similarly, a 10% duty cycle produced higher antibody titers than a 20% duty cycle in spite of again causing less skin damage [28]. Therefore, it is clear that the mechanisms of immune response by LFS are not well understood, and more research is needed in order to optimize protocols for transdermal vaccination using LFS.

4.2 Delivery of nanoparticles

In addition to the delivery of simple molecules, the delivery of nanoparticles and molecules in liposomal formulations has also been tested with LFS (Table 2). With respect to mechanistic investigations, quantum dots (QDs) have been utilized in several studies, because it is possible to tune their size and surface chemistry in a controlled fashion [63,71,72]. A recent study by Lopez *et al.* investigated the delivery of QDs (10 – 22 nm

Table 2. High molecular mass molecules (> 1000 g/mol) and particles that have been delivered transdermally with LFS.

Molecule	MM (kDa)	Ultrasound parameters				Co-enhancer	Skin model*	Ref.
		Frequency (kHz)	Amplitude (W/cm ²)	Skin-to-horn distance (m)	US ON time (min)			
Vasopressin	1.1	20	0.1 – 1	10	12 – 30	Hydrogel	In vitro	h HSS [84]
Cyclosporin A	1.2	20	0.4 – 1.2	5	5 – 30	Various	In vitro	Rat FTS [85]
LHRH	1.3	58	1.08	3	‡	1% SLS	In vitro	p FTS [32]
Octa-l-lysine-4-FITC [§]	2.5	20	2 – 50	2 – 4	0.05 – 3	None	In vitro	h HSS [73]
FITC-dextran [§]	4.4	41	0.06 – 0.30	3	120	None	In vitro	Rat FTS [86]
Inulin	5	58	1.08	3	‡	1% SLS	In vitro	p FTS [32]
Inulin	5	20	7.2	3	‡	1% SLS	In vitro	h FTS [61]
Inulin	5	20	7	10	¶	1% SLS	In vitro	p FTS [87]
Dextran	5	20	7	10	¶	1% SLS	In vitro	p FTS [87]
Insulin	5.8	20	0.0125 – 0.225	10	1 – 24	None	In vitro	h HSS [19]
Insulin	5.8	20	2.5 – 10	10	6 – 18	None	In vivo	Rat [88]
Insulin	5.8	20	0.173	5	60	None	In vitro	Rat [40]
Insulin	5.8	20	0.1	1	12	None	In vivo	h FTS [39]
Insulin	5.8	20	0.1	1	12	None	In vivo	Pig [35]
Insulin	5.8	20, 32	0.05	1	12	None	In vivo	Rabbit [36]
Insulin	5.8	20	0.1	1	12	None	In vivo	Rabbit [37]
Insulin	5.8	20	0.1 – 1	10	12 – 30	Hydrogel	In vitro	h HSS [84]
Insulin	5.8	48	#	2	5	None	In vivo	Mouse [23]
Insulin	5.8	105	#	2	45	None	In vivo	Rabbit [24]
Low-molecular-weight heparin	< 8**	20	7.0	10	¶	1% SLS	In vitro	p FTS [87]
Oligonucleotides	7 – 10**	20	2.4	33	10	1% SLS	In vivo	Rat [30]
Daniplestim	13	55	15	7.5	1.5	1% SLS	In vitro	p FTS [29]
IFN-γ	17	20	0.0125 – 0.225	10	24	None	In vivo	Rat [19]
Heparin	10 – 19**	20	7.0	10	¶	1% SLS	In vitro	h HSS [87]
FITC-dextran [§]	38	41	0.06 – 0.30	3	120	None	In vivo	Rat [86]
Erythropoietin	48	20	0.0125 – 0.225	10	24.0	None	In vitro	Rat FTS [19]
Poly-l-lysine-FITC [§]	51	20	2 – 50	2 – 4	0.05 – 0.5	None	In vitro	h HSS [73]
Bovine serum albumin	66	20	‡‡	5	2	0 – 1% SLS	In vitro	Rat FTS [27]
Dextran	70	58	1.08	3	‡	1% SLS	In vivo	Rat [32]
Dextran	70	20	7.0	10	¶	1% SLS	In vitro	p FTS [87]
Tetanus toxoid	150	20	2.4	5	1.3	1% SLS	In vitro	Mouse [31]
Tetanus toxoid	150	20	‡‡	7.5	0.75	0 – 1% SLS	In vivo	Mouse [28]

Table 2. High molecular mass molecules (> 1000 g/mol) and particles that have been delivered transdermally with LFS (continued).

Particles	Diameter	Ultrasound parameters				Co-enhancer	Skin model*	Ref.
		Frequency (kHz)	Amplitude (W/cm ²)	Skin-to-horn distance (mm)	US ON time (min)	Duty cycle		
Gold nanoparticles	5 nm	20	7.5	3	~3 ^{§§}	50%	<i>In vitro</i> p FTS	[63]
Iron oxide particles	5 – 10 nm	25	0.8	0	5	100%	<i>In vitro</i> p 700	[48]
Quantum dots	10 – 22 nm	20	7.5	3	~3 ^{§§}	50%	<i>In vivo</i> Mouse	[71]
Quantum dots	20 nm	20	7.5	3	~3 ^{§§}	50%	<i>In vitro</i> p FTS	[63]
Quantum dots	20 nm	20	2.4	5	3	50%	<i>In vitro</i> p 700	[72]
siRNA-liposome complex	50 nm	20	0.05	1	3	20%	<i>In vitro</i> Cell lines	[41]
Testosterone in solid lipid microparticles	~100 nm**	20	2.5 – 5	5	6 – 15	20 – 50%	<i>In vivo</i> Mouse	[89]
Lidocaine in polymer nanoparticles	200 nm	28	8	1	10	100%	<i>In vitro</i> Rat FTS	[90]
Liposomes	0.08 µm, 4.6 µm	20	##	5	2	50%	<i>In vivo</i> Rat	[27]
Microparticles	1 – 173 µm††	20	19	6	0.25 – 1.5	20 – 100%	<i>In vitro</i> Rat FTS <i>In vivo</i> Rat <i>In vitro</i> h HSS h FTS <i>In vitro</i>	[73]

*Legend: p: Pig, H: human, FTS: Full-thickness skin, HSS: Heat-stripped skin, and 700: 700-µm dermatomed skin.

†No treatment times were specified; see log *R* values in **Table 1** for relative extents of skin perturbation.

‡FTC: Fluorescein isothiocyanate.

§No treatment times were specified; skin was treated to a skin conductivity of 0.6 (kohm-cm²)⁻¹.

#Amplitude was not reported in W/cm²; reported intensity was 3000 – 8000 Pa.

**Molecular mass or size was not reported, but instead estimated from the paper's references.

††Amplitude was not reported in W/cm²; a setting of 20 – 30% on the machine was reported (VCX 500, Sonics and Materials, USA).

§§Approximate treatment time, skin was treated to attain a specified range of skin resistivity values.

¶¶Particles as large as 25 µm in diameter were observed to penetrate the skin, but larger particles did not.

LFS: Low-frequency sonophoresis, LHRH: Luteinizing-hormone-releasing hormone, MM: Molecular mass; SLS: Sodium lauryl sulfate.

in diameter), with varying surface charges (cationic, neutral and anionic) in order to evaluate LFS treatment to enhance skin penetration of transdermal carriers [71]. Lopez *et al.* found that the LFS treatment increased the amount of QDs penetrating past the epidermis by 500 – 1300%. Interestingly, however, the highest charged cationic QD did not penetrate the most, as originally expected, suggesting that there is an optimal cationic surface charge for designing transdermal carriers [71]. Other studies have considered the delivery of metallic nanoparticles into LFS-treated skin, including gold nanoparticles [63] and iron oxide nanoparticles [48]. The latter study, utilizing iron oxide nanoparticles, was conducted using zero clearance between the skin surface and the ultrasound horn, which is not typical of treatment protocols involving LFS. In general, it is advisable to leave some clearance between the skin and the ultrasound horn, even if it is a small distance (2 – 3 mm), to avoid the creation of pockets of air/coupling solution between the skin and the ultrasound horn. Without any clearance, small solution volumes trapped between the skin and the ultrasound horn, which are restricted from mixing with the bulk solution, could heat rapidly. This heating can cause thermal effects that are unaccounted for and could lead to difficulty in the interpretation of the mechanisms of enhancement.

Larger particles have also been delivered through LFS-treated skin, the majority of which are liposomal formulations. Liposomal formulations are a natural delivery vehicle in the context of LFS-mediated transdermal delivery because formulations of lidocaine within liposomes are already clinically used for topical anesthesia, which is discussed further in Section 5 (Table 3). An interesting application of liposomal formulations has been reported by Tran *et al.* for the delivery of siRNA-liposome complexes for treatment of melanoma [41]. The study showed that the application of LFS enabled the delivery of loaded cationic liposomes throughout the epidermis and the dermis of skin reconstructs, as well as in an *in vivo* mouse model, which led to a decrease of melanoma by a statistically significant amount [41]. Other studies have examined the delivery of liposomes or microparticles as large as 173 μm in diameter [27,73] and have shown that particles as large as 25 μm in diameter can penetrate the skin [73]. However, the study involving the delivery of microparticles as large as 173 μm was conducted at extremely high amplitudes, approximately three to sevenfold higher (19 – 49 W/cm^2) than the highest amplitudes commonly used for LFS-mediated transdermal delivery (7 – 8 W/cm^2). Therefore, although particles as large as 25 μm in diameter were delivered through heat-stripped skin, microscopy showed macroscopic holes in the skin [73], which casts significant concern with regard to the safety of this treatment protocol. Under typical LFS conditions, which have been tested for safety, it is generally believed that particles no larger than ~ 100 nm can penetrate LFS-treated skin as intact entities (Table 2).

5. Clinical applications of LFS

LFS has been utilized in many clinical applications for the delivery and extraction of different types of drugs and analytes (Table 3), in addition to being used for cosmetic purposes in skin rejuvenation and cellulite remediation. Here, we focus solely on clinical applications relevant to drug delivery and analyte extraction. The first clinical application of LFS involved the delivery of liposomal lidocaine, or lidocaine/prilocaine (both are small, hydrophobic molecules), to decrease the time of onset for local anesthesia [17]. This application has been well documented, with multiple pilot and clinical trials showing that LFS decreases the onset to anesthesia with lidocaine, from 30 to 60 min passively, to < 5 min with LFS pretreatment. This technology is FDA approved for use in both adults and children for local anesthesia prior to hypodermic injection, intravenous cannulation and blood donation (SonoPrep[®], Echo Therapeutics, Franklin, MA, USA, Figure 4) [16,17,74-76]. The device has also been tested for use in combination with iontophoresis, which results in an even shorter time to onset of anesthesia (2 min) [18]. Other applications of the device involve the extraction of interstitial fluid for blood glucose and lactate monitoring, which is currently in the process of attaining FDA approval [15,67].

Other studies have investigated the delivery of hydrophilic molecules in clinical applications (see log $K_{o/w}$ values in Table 3), including the delivery of histamine [77], kojic acid [78], ascorbic acid [78] and epinephrine [18]. Additionally, cyclosporine solution (molecular mass = 1203 kDa) has been utilized in combination with LFS for the treatment of alopecia areata [78]. In all cases, the drugs delivered were reported to have the desired effect. For complete experimental details of clinical trials, refer to Table 3.

6. Safety of LFS

The safety of LFS for use in animals and humans has been evaluated rather rigorously for single dose applications. Clinical studies with the SonoPrep device have shown that, following a single treatment (~ 10 s), there are usually minimal or no adverse reactions with the most common side effect being mild erythema [16,17,74-76]. As stated above, the SonoPrep has, therefore, been FDA approved for use in children and adults for decreasing the onset time to local anesthesia. The effect of LFS on animal models and cultured cell lines has also shown that the use of low ultrasound intensities is safe, causing no changes in skin pathology and cell viability [19,22,26,70,79]. In general, for single application treatments, thermal effects caused by LFS are of most consequence to the skin if not monitored properly, causing potentially serious side effects such as burns, epidermal detachment, epidermal or dermal necrosis and, on a cellular level, keratinocyte apoptosis [70,79]. However, these types of side effects are easily mitigated by minimizing the duration and intensity of the LFS treatment utilized.

Table 3. Drugs that have been tested in clinical (*in vivo* human) settings, including experimental details.

Drug	MM (Da)	log Ko/w*	Experimental details								Ref.		
			Equipment		Purpose	Metric utilized	Method		Participants			Results	
			Device	Manufacturer			Randomized	Blinded	Total	with LFS			
													Benefit
Electrical current	-	-	SonoPrep [‡]	Echo/Sontra [§]	Skin resistivity reduction	Increase in skin impedance	No	No	10	10	Yes	LFS led to an immediate decrease in skin impedance, which lasted for 24 h	[91]
Electrical current)	-	-	SonoPrep [‡]	Echo/Sontra [§]	Recovery time of skin barrier properties	Increase in skin impedance	No	No	10	10	Yes	LFS caused increased and sustained skin permeability for up to 48 h under occlusion. Skin barrier properties recovered rapidly after	[92]
Histamine	111	-0.70	Mésothérapie Ultrasonore [¶]	Transderma [#]	Transdermal histamine delivery with LFS	Papule formation caused by histamine, patient pain score	Yes	Yes	10	10	Yes	occlusion removal 90% of patients showed papule formation with LFS, compared to none without LFS. Pain scores with LFS were similar to pain scores for pin prick tests	[77]
Glucose extraction	180	-3.24	SonoPrep [‡]	Echo/Sontra [§]	Accurately monitor blood glucose through LFS-treated skin	Comparison of transdermal blood glucose readings to controls	No	No	10	10	Yes	Transdermal glucose monitoring was found to be safe and effective, with a correlation coefficient of 0.84 between the transdermal and control blood glucose measurements	[15]
Glucose extraction	180	-3.24	SonoPrep [‡]	Echo/Sontra [§]	Accurately monitor blood glucose through LFS and abraded skin	Comparison of transdermal blood glucose readings to controls	Yes	Yes	24	24	Yes	Good agreement was observed between the transdermal (both LFS and abraded skin) and control blood glucose measurements	[67]

*Log octanol–water partition coefficients reported from [65] (Syracuse Research Corp. Physical Property Database).

[‡]Operating frequency: 55 kHz; Amplitude ≤ 15 W/cm²; transducer-to-skin distance: 0.5 cm; duty cycle: 100%; coupling medium: 1.0% SLS and treatment times: ~10 s.

[§]Echo Therapeutics (Franklin, MA, USA) merged with Sontra in 2007.

[¶]Operating frequency: 36 kHz; Amplitude: 2.72 – 3.5 W/cm², transducer-to-skin distance: 0.5 cm; duty cycle: 29 – 38% coupling medium: saline and treatment time: 5 min.

[#]Transderma Systems (Tours, France).

**Ferndale Laboratories (Ferndale, MI, USA).

^{††}IontoCaïne (Abbot Laboratories, Chicago, IL, USA).

^{§§}EMLA: Eutectic mixture of local anesthetics.

i.v.: Intravenous; LFS: Low-frequency sonophoresis; MM: Molecular mass; NS: Not specified; SLS: Sodium lauryl sulfate.

Table 3. Drugs that have been tested in clinical (*in vivo* human) settings, including experimental details (continued).

Drug	MM (Da)	log Ko/w*	Experimental details						Ref				
			Equipment		Purpose	Metric utilized	Method			Participants		Results	
			Device	Manufacturer			Randomized	Blinded		Total	with LFS	Benefit	Conclusion
Liposomal lidocaine cream**	188, 176, 142	1.57, -1.85, -0.64	25 kHz, 50 – 100 mW/cm ²	NS	Treatment of melasma and solar lentigo	Skin color and luminosity index	NS	NS	48	32	Yes	Treatment with emulsion and LFS resulted in complete depigmentation in 75% of melasma cases and 43% total remission in solar lentigo cases	[78]
	234	2.44	SonoPrep [‡]	Echo/Sontra [§]	Ability of LFS to decrease onset of lidocaine to 5 min	Patients' pain scores during i.v. cannulation, skin irritation	Yes	Yes	94	45	Yes	91% of LFS-treated patients reported pain scores < 5, compared to 43% in the controls. Skin Irritation was similar in both	[16]
Liposomal lidocaine cream**	234	2.44	SonoPrep [‡]	Echo/Sontra [§]	Ability of LFS to decrease onset of lidocaine to 5 min in children	Children's and parents' pain scores with i.v. placement	Yes	Yes	77	38	Yes	Both children's (2.29 vs. 3.23) and parents' (2.47 vs. 3.39) pain scores decreased significantly with LFS/lidocaine treatment, compared to controls	[74]
Liposomal lidocaine cream**	234	2.44	SonoPrep [‡]	Echo/Sontra [§]	Ability of LFS to decrease pain associated with venipuncture in children	Children's' and parents' pain scores with i.v. placement	Yes	Yes	70	39	Yes	Compared to passive lidocaine for 30 min, 5 min of lidocaine post LFS resulted in statistically similar anesthetic effect for venipuncture	[76]
Liposomal lidocaine cream**	234	2.44	SonoPrep [‡]	Echo/Sontra [§]	Ability of LFS to decrease pain associated with blood donation	Patients' pain scores during blood donation, skin irritation	Yes	Yes	100	49	Yes	LFS was shown to decrease onset of anesthesia to 5 min in a safe manner for use with blood donation	[75]

*Log octanol–water partition coefficients reported from [65] (Syracuse Research Corp. Physical Property Database).

[‡]Operating frequency: 55 kHz; Amplitude ≤ 15 W/cm²; transducer-to-skin distance: 0.5 cm; duty cycle: 100%; coupling medium: 1.0% SLS and treatment times: ~10 s.[§]Echo Therapeutics (Franklin, MA, USA) merged with Sontra in 2007.[¶]Operating frequency: 36 kHz; Amplitude: 2.72 – 3.5 W/cm², transducer-to-skin distance: 0.5 cm; duty cycle: 29 – 38% coupling medium: saline and treatment time: 5 min.

#Transderma Systems (Tours, France).

**Ferndale Laboratories (Ferndale, MI, USA).

^{††}Intocaine (Abbott Laboratories, Chicago, IL, USA).^{§§}EMLA: Eutectic mixture of local anesthetics.

i.v.: Intravenous; LFS: Low-frequency sonophoresis; MM: Molecular mass; NS: Not specified; SLS: Sodium lauryl sulfate.

Table 3. Drugs that have been tested in clinical (*in vivo* human) settings, including experimental details (continued).

Drug	MM (Da)	log Ko/w*	Experimental details						Ref.
			Equipment		Purpose	Metric utilized	Method		Results
			Device	Manufacturer			Randomized	Blinded	
Lidocaine, epinephrine [‡]	234, 183	2.44, -1.37	SonoPrep [†]	Echo/Sontra [§]	Ability of LFS, in combination with iontophoresis, to decrease onset to 2 min	Patients' pain scores on needle prick	Yes	Yes	Pain scores for 10 min iontophoresis and 2 min iontophoresis, following LFS, were statistically similar, showing that LFS decreases onset time to 2 min
									[18]
Lidocaine, prilocaine (EMLA cream ^{§§})	234, 220	2.44, 2.11	SonoPrep [†]	Echo/Sontra [§]	Ability of LFS to decrease onset of EMLA cream to 5 min	Patients' pain scores on needle prick	Yes	Yes	Onset of cutaneous anesthesia was decreased from 60 min (without LFS) to 5 min with LFS, as evidenced by statistically similar pain scores
									[17]
Methylprednisolone ointment	374	1.82	25 kHz, 50–100 mW/cm ²	NS	Treatment of alopecia areata	Hair re-growth (total, partial, none)	NS	NS	29% of patients had total re-growth and 57% of patients showed partial re-growth of hair
									[78]
β-Methasone-17-valerate cream	477	3.60	Mésothérapie Ultrasonore [¶]	Transderma [#]	Efficacy of topical steroids for vasoconstriction	Color of skin evaluated by chromometer and visual inspection	Yes	Yes	Vasoconstriction increased after LFS and persisted for 6 h, but disappeared by 24 h. Occlusion increased the effect of LFS on vasoconstriction
									[93]
Cyclosporine solution	1203	2.92	25 kHz, 50–100 mW/cm ²	NS	Treatment of alopecia areata	Hair re-growth (total, partial, none)	NS	NS	34% of patients had total re-growth and 33% of patients showed partial re-growth of hair within 3 months
									[78]

*Log octanol–water partition coefficients reported from [65] (Syracuse Research Corp. Physical Property Database).

[†]Operating frequency: 55 kHz; Amplitude ≤ 15 W/cm²; transducer-to-skin distance: 0.5 cm; duty cycle: 100%; coupling medium: 1.0% SLS and treatment times: ~10 s.

[§]Echo Therapeutics (Franklin, MA, USA) merged with Sontra in 2007.

[¶]Operating frequency: 36 kHz; Amplitude: 2.72 – 3.5 W/cm², transducer-to-skin distance: 0.5 cm; duty cycle: 29 – 38% coupling medium: saline and treatment time: 5 min.

[#]Transderma Systems (Tours, France).

**Ferndale Laboratories (Ferndale, MI, USA).

[‡]Lidocaine (Abbott Laboratories, Chicago, IL, USA).

^{§§}EMLA: Eutectic mixture of local anesthetics.

i.v.: Intravenous; LFS: Low-frequency sonophoresis; MM: Molecular mass; NS: Not specified; SLS: Sodium lauryl sulfate.



Figure 4. Low-frequency ultrasound treatment in a clinical setting with the SonoPrep® device (Echo Therapeutics, Franklin, MA, USA). The patient holds a conductive polymer hand-piece and the device automatically stops when the skin impedance is decreased to the desired treatment level. Usual treatment times are on the order of 10 s (reprinted with permission from [92]).

Although LFS has been generally accepted as safe for single dose uses, there have been no significant studies of the prolonged or repeated use of such treatments. These types of studies will need to be conducted to establish the safety of LFS in the treatment of chronic diseases. For example, the current application of non-invasive blood glucose monitoring would require daily LFS treatment, as would other drug delivery applications. Therefore, sustained *in vivo* safety studies are needed to understand the potential skin toxicities involved with repeated LFS treatment.

7. Conclusion

LFS has been shown to allow transdermal delivery of both hydrophilic and high-molecular mass permeants at therapeutic levels. LFS is known to increase the permeability of skin through the process of acoustic cavitation above the skin, which causes the formation of acoustic microjets on the surface of the skin in a non-uniform manner. The heterogeneous occurrence of cavitation leads to the formation of LTRs, which have much higher permeability than the surrounding non-LTRs. When a chemical enhancer, such as the surfactant SLS, is included in the treatment of skin with LFS, a very strong synergistic enhancement in skin permeability is observed. This enhancement in skin permeability allows delivery of hydrophilic permeants, whose transport through the skin can be explained in the context of the APPM. Additionally, LFS-mediated transdermal delivery can also be utilized to deliver macromolecules, including proteins, hormones, biopolymers, fibers, vaccines, liposomes and even nanoparticles. This technology has been tested in clinical trials and is

currently FDA approved for use with local anesthetics. In addition, the technology has the potential to deliver a broad class of drugs in a manner that avoids side effects associated with oral delivery and transcutaneous injection. However, further research is necessary to better understand and control the reproducibility and safety of the ultrasound parameters used in clinical treatment. A potential exciting area of future research involves LFS-mediated transdermal vaccination because it has been shown that LFS itself can act as a physical adjuvant.

8. Expert opinion

The use of LFS has shown great promise in the transdermal delivery of therapeutics, including hydrophilic drugs and macromolecules. Since the initial investigations of transdermal delivery utilizing ultrasound frequencies between 20 and 100 kHz 2 decades ago, this technology has already spawned a number of startups, received FDA approval for the topical delivery of local anesthetics and is currently being investigated for other clinical applications, such as skin permeabilization for non-invasive blood glucose monitoring. With this strong beginning, LFS-mediated transdermal delivery has the potential to gain an even stronger clinical foothold by exploiting the unique strengths of this technology. For example, LFS can be utilized for systemic, regional and local delivery with the ability of controlling the transdermal delivery profile of the active therapeutic compound by varying the ultrasound treatment parameters. Therefore, in addition to the systemic delivery of proteins, such as insulin, local delivery of drugs to treat skin disorders may be an area where LFS can exploit its ability to permeabilize skin in a controlled manner. This may be particularly relevant to skin disorders where the delivery of active therapeutics through the skin represents a limiting transport step, and the number of side effects can be limited by localized therapy, such as in the case of psoriasis plaques [80,81].

Another application where LFS may be able to distinguish itself from other physical skin permeability enhancers is in the development of a closed-loop system for the monitoring of blood analytes and the subsequent delivery of appropriate therapeutics. The advancement of the cymbal array device by Smith *et al.* [40] has now opened the door to a wearable LFS device. Additionally, the feasibility of LFS for non-invasive blood glucose monitoring has already been established [15,21,67]. Therefore, the technology necessary to create such a device already exists. Nevertheless, many obstacles still need to be overcome before a device is ready for use in the clinic. For example, potential challenges include the development of efficient algorithms involved in sensing and delivery (because there is a lag-time associated with both transdermal blood glucose monitoring and the transdermal delivery of insulin, accurate predictions will be crucial) and in the design of a battery system that would be powerful enough to drive the LFS cymbal array, while still being small enough to allow for a wearable device.

A final exciting area where LFS could have a significant impact is in the field of transdermal vaccination. The feasibility of transdermally delivering high-molecular mass vaccines, such as TT, by LFS has already been established in *in vitro* and *in vivo* animal models [28,31]. It is also known that targeting of the Langerhans cells in the skin can result in an enhanced immune response with respect to vaccine injection [68,82]. However, in addition to allowing increased amounts of antigens to reach the Langerhans cells by increasing skin permeability, the LFS treatment itself has been shown to activate the Langerhans cells and elicit an immune response [31,70]. In fact, Tezel *et al.* have shown that the immune response induced by the delivery of 1.3 µg of TT to the Langerhans cells by LFS is equal to that of 10 µg of TT injected subcutaneously in mouse models [31]. Clearly, more research is needed to fully understand, characterize and control this effect. This would involve not only studying delivery of antigens and the resulting immune response, but also the formulation of vaccines for delivery via the transdermal route. An additional variable that could also be studied is the addition of a co-enhancer during the LFS treatment because it has been shown that the immune response elicited by TT can increase with the inclusion of SLS in the LFS coupling medium, although the response is not directly proportional to the SLS concentration [28].

In addition to the specific areas discussed above, additional research is needed on the general safety of the technology. If a closed-loop device for glucose monitoring and insulin delivery is developed, long-term safety studies are necessary to determine whether repeated treatments with LFS are safe. Current safety studies have only been conducted for relatively short times (a maximum of 24 – 48 h), and usually following only a single LFS treatment. Moreover, if drugs are to be systemically and repeatedly delivered by LFS, more research is needed into the reproducibility of skin permeability induced by the LFS treatments, both between different patients and after repeated treatments in a single patient. Additionally, further research on the generation of more uniform skin permeability enhancement would greatly benefit LFS treatments. Under current treatment protocols with SLS, only 5 – 25% of the skin surface treated by LFS contains LTRs, which are

the skin regions through which the majority of transdermal transport occurs (Figure 3) [57]. If one could increase the size of the LTRs to cover the entire skin surface treated, the area of skin treatment sites could decrease by up to 20-fold. This would result in decreased power needed to operate the LFS device and aid in further miniaturization of a clinical device. For example, Paliwal *et al.* have recently shown that an aqueous mixture of 0.5% 3-(decyl dimethyl ammonio) propane sulfonate and polyethylene glycol dodecyl ether induced three times more LTR area than 1% SLS [83]. Therefore, research on cavitation enhancers for use with LFS, including more potent synergistic chemical enhancers, deserves further investigation.

In conclusion, LFS-mediated transdermal delivery of hydrophilic drugs and macromolecules shows great promise to address key needs in the medical field, such as developing a closed-loop system for tighter glycemic control, treating localized skin disorders and transdermal vaccination. However, there are still opportunities to make technological and mechanistic advances with this technology that can increase its clinical utility. To reach the ultimate goal of positively affecting patients' lives, LFS researchers must focus on the unique advantages offered by LFS, including exploiting these advantages when investigating new treatments involving LFS.

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Declaration of interest

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Affiliation

Baris E Polat, Daniel Blankschtein[†] & Robert Langer

[†]Author for correspondence

Massachusetts Institute of Technology,
Department of Chemical Engineering,
Room 66 – 444,

77 Massachusetts Avenue,

Cambridge, MA 02139, USA

Tel: +1 617 253 4594; Fax: +1 617 252 1651;

E-mail: dblank@mit.edu