

● *Original Contribution*

ULTRASOUND-MEDIATED TRANSDERMAL TRANSPORT OF INSULIN *IN VITRO* THROUGH HUMAN SKIN USING NOVEL TRANSDUCER DESIGNS

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Abstract—Recent studies have shown that ultrasound (US)-mediated transdermal drug delivery offers a promising potential for noninvasive drug administration. The purpose of this study was to improve low-frequency (20 kHz) US methods for enhancing the transport of insulin *in vitro* across human skin. The feasibility of using US produced by small, lightweight novel transducers was explored for enhancing the transport of insulin across skin. Previous investigators have used US devices such as large, heavy sonicators or commercially obtained transducers for this type of research. The experiments carried out in this study used two low-profile novel US transducer arrays, the stack and standard array, for improved transport of insulin. The stack array generated a spatial peak temporal peak intensity (I_{SPTP}) of $15.4 \pm 0.6 \text{ mW/cm}^2$ and the standard array had an I_{SPTP} of $173.7 \pm 1.2 \text{ mW/cm}^2$. Spectrophotometric absorption techniques were used for determining insulin transport *in vitro* across human skin. Compared with passive transmission ($4.1 \pm 0.5 \text{ U}$) over an exposure period of 1 h, the standard array facilitated over a sevenfold increase in the noninvasive transdermal transport of Humulin[®] R insulin ($45.9 \pm 12.9 \text{ U}$). Using Humalog[®] insulin with the standard array, there was a fourfold increase in the US-facilitated transmission over that in the control. These promising results indicate that low-frequency US can be used in a practical device for enhanced transport across the stratum corneum. (E-mail: nbs@enr.psu.edu) © 2003 World Federation for Ultrasound in Medicine & Biology.

Key Words: Ultrasound, Transdermal, Drug delivery, Insulin, Skin.

INTRODUCTION

Recent studies have shown that ultrasound (US)-mediated transdermal drug delivery offers promising potential for noninvasive drug administration (Mitragotri et al. 1995, 1996; Zhang et al. 1996; Johnson et al. 1996; Mitragotri and Kost 2000). There exist several known methods of transdermal drug delivery, such as chemical mediation (liposomes and chemical enhancers) and physical mechanisms (iontophoresis, electroporation and US, also called sonophoresis or phonophoresis) that are methods of enhancement (Prausnitz 1997, 1999; Montorsi et al. 2000). In the past, US has been used by physical therapists and physicians to treat patients with local musculoskeletal inflammation using topically ap-

plied steroids (Byl et al. 1993). More recently, the use of US was being explored for chemical activation of drugs by for treatment of cancers (sonodynamic therapy). For example, US energy can enhance effects of thrombolytic agents such as urokinase (Tachibana and Tachibana 1998).

The focus of this research was to develop a practicable method for drug delivery using low-frequency (20 kHz) US to enhance the transport of insulin across skin using novel transducer designs. Of note, high-frequency US in the MHz range has been used for transdermal delivery of various proteins and drugs. Previous attempts to use high-frequency US ($\approx 1 \text{ MHz}$ and ≈ 1 to 3 W/cm^2) to enhance transdermal drug delivery have produced inconsistent results that were found to vary significantly from drug to drug (Bommanna et al. 1992a, 1992b; Mitragotri et al. 1997). Pulsed US at 1 MHz has been used to increase transdermal absorption of indomethacin from an ointment in rats (Asano et al. 1997).

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Also, the combination of chemical enhancers and therapeutic US (1 MHz, 1.4 W/cm², continuous wave, CW) on transdermal drug transport have been investigated with some success (Johnson et al. 1996). Other high molecular weight proteins were also shown to have increased transdermal permeation in the presence of US. US at 1 MHz has been shown with rats and guinea pigs to increase skin permeation of D-mannitol, a highly polar sugar alcohol, inulin, a high molecular weight polysaccharide and physostigmine, a lipophilic anticholinesterase drug (Levy et al. 1989).

The difference between high- (1 to 3 MHz) and low- (\approx 20 kHz) frequency US appears to be that low-frequency US enhances transdermal drug transport 1000 times greater than does high-frequency US (Mitragotri et al. 1996). The hypothesis for the physical mechanism is that low-frequency US enhances transdermal transport through aqueous channels in the stratum corneum that are generated by the distortion of the lipid bilayer due to cavitation.

However, the physical mechanism of the enhancement using US is far from being fully understood. Some researchers have concluded that, at 168 kHz using CW US and at 1.9×10^5 Pa, US induced a new structural state and generated defects in human stratum corneum specimens. They suggest that the dimensions of the defects (20 μ m) were large enough to allow the transdermal passage of high molecular weight drug molecules that normally elude unenhanced transdermal drug delivery (Wu et al. 1998).

Transport of both vasopressin and insulin across human skin *in vitro* has been demonstrated using a 20-kHz sonicator (Sonicator W385, Heat Systems, Farmingdale, NY) over a period of 5 h using an intensity as low as 100 mW/cm² (Zhang et al. 1996). From *in vitro* human skin and *in vivo* rat experiments, the transdermal transport of insulin has been shown using a 20-kHz US sonicator operating at intensities from 12.5 to 225 mW/cm² (Mitragotri et al. 1995).

The purpose of this research was to explore the feasibility of using US produced by novel transducers for enhancing the transport of insulin across skin *in vitro*. Previously, researchers have demonstrated the feasibility of transdermal insulin delivery using sonicators or commercially obtained transducers. With respect to exposimetry, many investigators who have used sonicators to demonstrate transdermal drug delivery only report a value for the ultrasonic intensity, without a description of how it was determined. As shown above, much of the drug delivery literature reports intensity values, but without indicating essential information, such as whether it was the spatial peak temporal peak or temporal average (IEEE 1990). The goal of this research was to introduce a practica-

ble (*i.e.*, low-cost, light weight) US device to noninvasively transport insulin, a large molecular weight protein (MW = 5807.69 Da), across skin *in vitro*. To accomplish this task, this research explored the use of the cymbal transducer as a single element or configured as an array for transdermal insulin delivery, along with accurately quantifying the acoustic field.

MATERIALS AND METHODS

Ultrasound transducers: cymbal transducer, standard array and stack array

Three low-frequency US designs based on the "cymbal" transducer were used for these insulin transmission experiments. The design, fabrication and electrical characteristics of these transducers have been described in detail elsewhere and will be discussed herein briefly (Newnham et al. 1991, 1994; Dogan et al. 1997; Newnham and Dogan 1998; Tressler et al. 1998). The single-element transducer, designated the "cymbal" transducer, was made of PZT-4 piezoelectric material (lead zirconate-titanate, Piezokinetics, Inc., Bellefonte, PA) and had a frequency range of 20 to 50 kHz. Design of the cymbal consists of a piezoelectric disk placed between two titanium caps with air cavities beneath the caps (Fig. 1a). The ceramic disk, with a diameter of 12 mm and 1-mm thickness, resonated in the radial mode. Due to the radial oscillations of the disk (*i.e.*, vibrations move from the center of the disk to the edges with radial symmetry) the presence of the cavities allowed for the conversion of the radial displacements into large axial displacements normal to the cap surface. For driving the transducer, a coaxial cable was attached to the transducer and enclosed in Uralite[®] polymer (FH 3550, H.B. Fuller, St. Paul, MN). The final shape of the transducer with cylindrical plastic housing was 18 mm in diameter and 29-mm long (Fig. 1b). For the array, four transducers were connected in parallel and encased in Uralite[®] to produce a transducer array arrangement. The ceramics and physical size of the elements were identical to those of the cymbal transducer. A two-by-two elemental pattern was used for the four transducers; the final array was in a $37 \times 37 \times 7$ mm³ block (Fig. 2).

The block diagram for the driving equipment for the cymbal and array transducers is shown in Fig. 3. The radiofrequency (RF) signal driving the transducer was generated by a frequency pulse/function generator (Model 393, Wavetek Inc., San Diego, CA) and amplified by an RF amplifier (Model 40A12, Amplifier Research, Souderton, PA). The electrical impedance of each device was tuned to the output impedance of the amplifier by an external LC (L = inductor, C = capacitor) network. A series inductor of 33 mH was used to tune the single

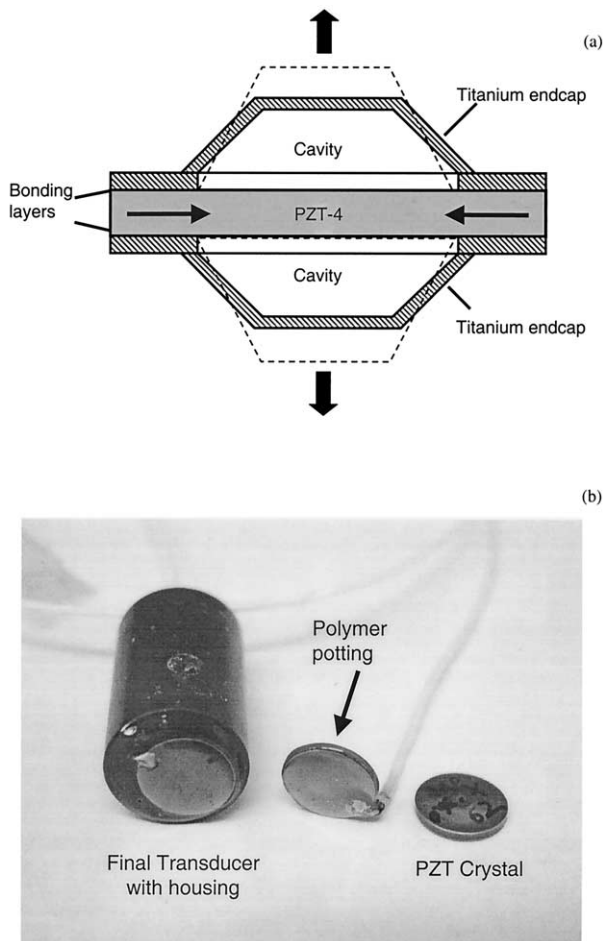


Fig. 1. (a) The single-element transducer, designated the “cymbal” transducer, made of piezoelectric material PZT-4 (lead zirconate-titanate), operated at a frequency of 20 kHz. The cymbal disk was placed between two titanium caps with air cavities beneath the caps that give rise to radial oscillations of the disk. (b) View of (right) a 12-mm diameter cymbal transducer, that was connected with a coaxial cable and housed in (center) polymer material to ensure electrical insulation between the end caps. The transducer was encased in (left) a housing for stability and positioning in water.

element cymbal close to the desired frequency. For the standard array, a π network was used with a series inductor of 4.6 mH and two parallel reactors (9.1 nF and 3.9 mH). The stack array used a t-network with L series = 4.6 mH and C parallel = 11.7 nF. Pulse period, duty cycle and exposure time of the RF signal from the frequency generator was monitored using an oscilloscope (Tektronix 2213A, Beaverton, OR).

For the *in vitro* experiments, the signal generator operating at 20 kHz had a 1 V (peak-to-peak) output with pulse duration of 200 ms and pulse repetition period of 1 s (*i.e.*, 20% duty cycle); the amplifier gain was set to 50 dB.

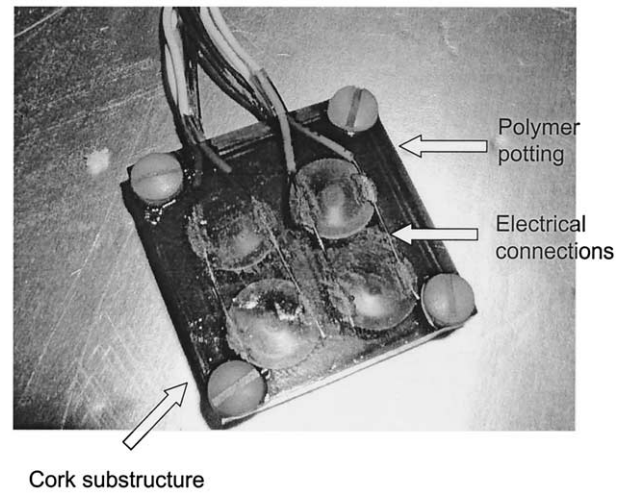


Fig. 2. Standard array made up of four cymbal transducers, connected in parallel, encased in Uralite® polymer and arranged in a two-by-two elemental pattern. Dimensions of the array were $37 \times 37 \times 7 \text{ mm}^3$.

Exposimetry of the cymbal transducer, stack array and standard array

For determining the intensity at a plane 1 mm from the transducer face, the ultrasonic intensities from the cymbal transducer, standard array and stack array were measured with a 9-mm diameter, calibrated ($-212.25 \text{ dB re } 1 \text{ V}/\mu\text{Pa}$) miniature omnidirectional reference hydrophone (Model TC4013, S/N: 5199093, Reson, Inc., Goleta, CA) in a $51 \times 54 \times 122 \text{ mm}^3$ partially anechoic tank containing degassed distilled water. A computer-

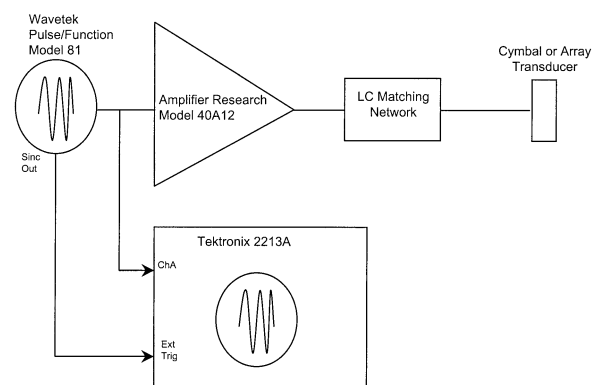


Fig. 3. Experimental setup of the function generator and the amplifier used for driving the cymbal single-element transducer and four-element array. The three transducers were electrically driven under similar conditions. The pulsed signal ($f = 20 \text{ kHz}$) from the signal generator was at 1 V_{pp}, pulse duration 200 ms, pulse-repetition period 1 s (*i.e.*, 20% duty cycle). Amplifier gain was 50 dB. Intensity results in mW/cm^2 were determined over a plane 1 mm from the transducer face.

controlled exposimetry positioning system was used for automated scanning. The scanning step size for each device was 1 mm, but the scanning area was different for the cymbal and arrays due to their different sizes. The scanning areas were 30, 40 and 100 mm² for the cymbal transducer, stack array and standard array, respectively. Spatial peak-temporal peak intensity (I_{SPTP}) and spatial peak-pulse average (I_{SPPA}) were determined over a plane 1 mm from each transducer face using the hydrophone based on three to five scanings of each transducer for a mean and SD of the results (IEEE 1990; AIUM 1998).

Skin handling

Samples of whole human skin were obtained from two reputable sources: Ohio Valley Tissue and Skin Center (Cincinnati, OH) and Intermountain Tissue Center (Salt Lake City, UT). To ensure safety, skin samples were tested by the skin banks to be negative for infectious disease before shipment to the Pennsylvania State University. The skin handling and usage protocol was approved by the University Biosafety Committee and processed according to the Pennsylvania State University Safety Guidelines regarding use of human tissue. Upon receiving the skin packed in dry ice, the skin was kept in a -70°C freezer until it was ready to be used. Detailed records of the age, gender, race, skin location and cause of death were documented. Human skin from the abdomen was used for determining enhanced transport of insulin using US.

Franz diffusion cell

A custom-made Franz diffusion cell (Fig. 4a) was designed and specially fabricated for this project (A.B. Seal Glassblowing, Bellefonte, PA). The cell consisted of an upper donor compartment and lower receiver compartment with a 100-mL volume. With three sampling ports for pipette removal of solution from the lower receiver compartment, the inside diameter of the sample port was 13 mm. Transmission experiments placed the stratum corneum or skin surface of the whole skin facing toward the donor compartment. A horseshoe clamp was used to secure the skin and the two compartments along with an o-ring to aid in securing the skin with the pinch clamp. Although the cell had a 50-mm i.d. hole in both the upper and receiver compartments, the opening of the skin was limited to 31 mm by two Plexiglas rings that gently held the skin. The clamp held the compartments along with the skin, o-rings and Plexiglas rings.

A uniform mixture was maintained using a magnetic stirring bar placed at the bottom of the receiver above a stirrer (Model PC-210, Corning, Acton, MA), with all experiments performed at room temperature. A photograph of the actual cell is shown in Fig. 4b.

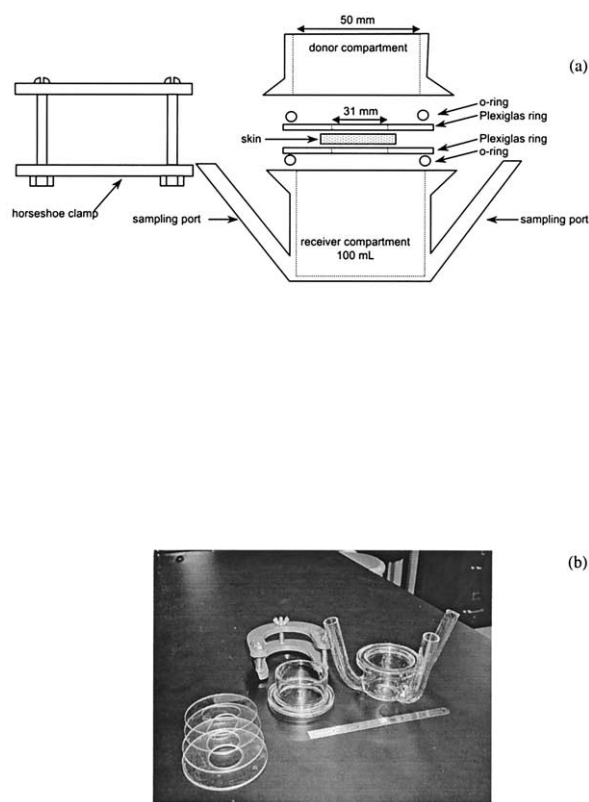


Fig. 4. (a) The Franz diffusion cell designed and specially fabricated for this project (not to scale), showing the upper donor compartment and lower receiver compartment (100 mL). The skin surface was placed facing toward the donor compartment. A horseshoe clamp secured the skin and the two compartments and an o-ring aided in securing the skin with the pinch clamp. (b) Photograph of the Franz diffusion cell.

Spectrophotometer calibration

Absorbance measurements using a spectrophotometer (Model UV VIS 160 M, Shimadzu, Columbia, MD) were used for determining the enhancement of insulin delivery across skin using US. US-mediated transport was determined using both Humulin[®] R regular insulin and Humalog[®] insulin (rDNA U-100, Eli Lilly and Co., Indianapolis, IN). Various concentrations of Humulin[®] R and Humalog[®] insulin were prepared for generating a calibration curve. Doses of insulin are measured in units (U) and Humulin[®] R and Humalog[®] insulin both contain 100 U/mL in a 10-mL bottle (Humulin 1999).

Insulin and saline (10 mM phosphate-buffered saline, PBS, catalog No. P-3813, Sigma, St. Louis, MO) concentrations of 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5 and 6.5 U/mL were prepared for absorbance measurements at 276 nm. A saline/insulin mixture at the proper concentration was stirred for 20 to 30 min before transfer to a quartz cuvette with a nominal volume of 1.4 mL and 10-mm path length (Model 9BQ10, Starna

Table 1. Intensity results

| Intensity (mW/cm ²) | Single-element cymbal | 2 × 2 Stack array | 2 × 2 Standard array |
|---------------------------------|-----------------------|-------------------|----------------------|
| I _{SPTP} | 0.522 ± 0.026 | 15.398 ± 0.600 | 173.669 ± 1.183 |
| I _{SPPA} | 0.006 ± 0.001 | 0.152 ± 0.024 | 1.100 ± 0.047 |

The three transducers were electrically driven under similar conditions. The pulsed signal ($f = 20$ kHz) from the signal generator was at 1 V_{pp}, pulse duration 200 ms, pulse-repetition period 1 s (*i.e.*, 20% duty cycle). Amplifier gain was 50 dB. Intensity results mW/cm², determined over a plane 1 mm from the transducer face.

Cell, Inc., Atascadero, CA). The cuvette was transferred to the spectrophotometer for absorption measurements at the proper wavelength. The calibration curve for the skin experiments was plotted as the absorbance vs. the insulin (U/mL) for each wavelength. Regression analysis of the absorbance based on insulin concentrations generated a linear regression equation along with the R² values of the equation.

Statistical analysis was performed using Microsoft Excel® (Microsoft Corp, Redmond, WA). ANOVA was used to analyze the statistical significance of the differences among the means of groups. The F value was used to determine if the between-group differences were significantly greater than chance. For all the data presented, a double asterisk was used if the F value was less than the 0.01 level of significance.

Insulin transmission through skin in vitro

For maintaining constant humidity and temperatures, experiments were performed in a custom-built 61 × 10⁻¹ × 122 cm³ box lined with insulation. To prevent the skin from drying, the humidity within the box was maintained at 75% using a humidifier (Holmes HM-725, Milford, MA). The evening before an experiment, the PBS was prepared and degassed under a high vacuum to remove dissolved oxygen.

At the beginning of an experiment, the skin was removed from the -70°C freezer and allowed to warm to room temperature. The skin temperature was monitored using a digital thermocouple (Fluke 77DVM, 80TK, 80PK-1, Fluke Corporation, Everett, WA). The skin was mounted into the Franz diffusion cell with the stratum corneum side facing the upper or donor compartment. After the receiver compartment was filled with saline, the compartments were clamped together. Care was taken to remove all bubbles from the receiver and ensure complete contact between the saline and the skin. A uniform mixture was maintained using a magnetic stirring bar, with all experiments performed at room temperature.

For the donor compartment, 10 mL of Humulin® R or Humalog® 100 U/mL insulin was diluted with 10 mL of saline to produce a total volume of 20 mL with a 50-U/mL insulin concentration. The insulin concentration was placed into the donor chamber with the face or

tip of the transducer submerged under the saline approximately 5 mm above the skin. After 1 h of either a control or US experiment, exposure was terminated and the concentration of insulin in the receiver chamber was determined using the spectrophotometer. Absorbance measurements were performed on three to eight samples removed from the receiver chamber at each wavelength. The mean and SD ($\bar{x} \pm SD$) at each wavelength were determined. Using the calibration curve of insulin and saline and knowing the volume of the receiver cell, the transmission of insulin over a period of 1 h was calculated for the skin.

RESULTS

Exposimetry of the cymbal transducer, standard and stack array

Using similar driving conditions, the intensity was determined in a plane 1 mm from each transducer face. All three devices (*i.e.*, cymbal transducer, standard array and stack array) were driven with a 1 V_{pp} signal with a 20% duty cycle amplified by 50 dB with a 1-s pulse-repetition period. Each device was scanned 3 to 5 times to produce a mean and SD of the intensity results. Table 1 lists the mean and SD ($\bar{x} \pm SD$) of the intensity results for the cymbal transducer, standard array and stack array. From the table, the standard array had the largest I_{SPTP} and I_{SPPA} values in the plane 1 mm from the face of the transducer compared with those of the single-element cymbal transducer and the stack array under similar driving settings.

Insulin transmission through skin in vitro

In vitro, human skin samples from the abdominal region were used for determining enhanced transport of insulin by US using either the single-element cymbal transducer, stack array or standard array. Spectrophotometer calibration results for the absorbance vs. Humulin® R or Humalog® insulin concentration (U/mL) produced a linear regression with an R² no less than 0.985. After exposure to either a control or US setting for 1 h, samples from the receiver compartment were placed in the spectrophotometer and the absorbance was recorded. Using the calibration curve, the amount of insulin trans-

Table 2. Insulin delivery

| Insulin units | Humulin® R | Humalog® |
|-----------------------|-----------------------|----------------------|
| Control | 4.1 ± 0.5 (n = 3) | 7.0 ± 4.4 (n = 5) |
| Single-element cymbal | 7.4 ± 3.1 (n = 2) | (not performed) |
| Stack array | 20.3 ± 9.3* (n = 3) | 19.9 ± 14.4* (n = 2) |
| Standard array | 45.9 ± 12.9† (n = 15) | 30.8 ± 12.6‡ (n = 6) |

A total of 36 abdominal skin samples were used *in vitro* for 23 Humulin® R and 13 Humalog® insulin-transmission experiments. Results in the U of insulin delivered over 1 h. %*F value > 0.05 level of significance; †F value > 0.01.

ported across the skin over a period of 1 h was determined. Table 2 lists the experimental results of the insulin transport under the control and US conditions using three transducers. Data are listed as the mean and SD ($\bar{x} \pm SD$), along with the number of experimental trials (n value) for each skin sample. Compared to the control experiment, an ANOVA found the use of US for transdermal transport of insulin, whether it was with the single-element cymbal transducer, stack array or standard array, to be statistically significantly different.

A gross examination of the skin was performed after exposure to detect for visible lesions on the skin surface. Visual and microscopic examination of the post-US exposed skin did not produce any noticeable damage or significant change to the skin.

DISCUSSION

Past research has demonstrated the possibility to deliver and control therapeutic doses of proteins such as insulin, interferon gamma, and erythropoietin across human skin using US (Mitragotri et al. 1995). Other researchers have investigated the *in vitro* penetration and the *in vivo* transport of flufenamic acid in skin with US (Hippius et al. 1998). In the flufenamic acid study, US exposure was from 5 to 30 min with intensities up to 1.5 W/cm². Although there was a pronounced effect of US on the transmembrane absorption of the drug, there was also a rise of temperature up to 4.5°C. US at 1 MHz has also been used to enhance the transdermal absorption of indomethacin, studied in rats using intensities from 0.25 to 1 W/cm². The researchers reported no significant skin temperature rise and no notable damage to the skin; however, damage was noted as the intensity and the time of application of US increased beyond 1 W/cm² (Miyazaki et al. 1992).

The goal of this research was to determine if a US device based on the low-profile cymbal transducer device could be used for ultrasonic transdermal insulin transport. Sonicators have been shown to transdermally deliver insulin across skin *in vitro* and *in vivo* using intensities as low as 12.5 mW/cm² (Mitragotri et al.

1995). Based on the I_{SPTP} results, the standard array produced intensities that have previously been shown to transdermally deliver insulin across skin using a sonicator. Although a commercial sonicator has been an excellent device for demonstrating drug delivery, the ultrasonic probe or converter from a commercial sonicator can weigh almost a kg or more, but the standard array weighs less than 22 g. From Table 1, the results indicate that the standard array had the highest I_{SPTP} and I_{SPPA} intensities for similar driving conditions used for the transmission experiments.

From Table 2, the Humulin® R results indicate an increase in the transmission of insulin using US compared to passive transmission (*i.e.*, control conditions of no US). Over a 1-h period using the single-element cymbal transducer with Humulin® R, almost twice the amount of insulin was transported across the skin *via* US (7.4 U) compared to the amount with the control (4.1 U). With Humulin® R, use of the stack (20.3 U) and standard (45.9 U) US arrays resulted in a four- or sevenfold increase, respectively. Although the passive transmission (control, 7.0 U) across the skin of Humalog® was slightly higher than with Humulin® R, US again increased the transmission of the insulin. Based on the Humulin® R single-element results, along with the expense of human skin, Humalog® experiments using the single element were intentionally not performed in preference to using the arrays. From the table, more than twice the amount of Humalog® was transmitted across the skin using the stack array (19.9 U), and the standard array had a greater effect on transmission (30.8 U). For both types of insulin, compared to the control results, the use of the arrays for transmission of insulin was statistically significantly different at a *p* value of 0.05 or better.

Although not presented here as a formal study, preliminary tests have been performed on the postsonicated insulin using the standard array to determine possible damage or degradation of the insulin molecule. Samples of the insulin were sent to a laboratory (Celsis Laboratory Group, Edison, NJ) to determine the integrity of the molecule using high-performance liquid chromatography (HPLC). Inspection of the chromatograms between the exposed and unexposed samples indicated that the insulin did not break down after sonication; however, further research using the cymbal transducer will explore this issue in greater detail.

For a typical diabetic patient weighing 70 kg, approximately 12 U of insulin is taken 3 times each day for a total of 36 U per day (Krall 1988). Based on these results, the standard array can deliver 30 to 45 U per h across human skin *in vitro*; therefore, one dose might be delivered in less than 30 min, depending on the type of insulin.

Specifically for insulin, there has only been limited research, although positive results have been shown for the papers listed (Tachibana and Tachibana 1991, 1992;

Mitragotri *et al.* 1995; Zhang *et al.* 1996; Boucaud *et al.* 2000) (Table 1). Over a frequency range of 20 to 105 kHz, enhanced transport in the presence of US has been shown in both *in vitro* and *in vivo* experiments. Yet all of the experiments were performed using either a US sonicator, ultrasonic bath or commercial transducer. The major drawback so far in exploiting US for noninvasive drug delivery has been the large size and poor mobility of sonicators.

Visual and microscopic examination of the US-exposed skin did not reveal any noticeable damage to the skin. Other researchers have reported noticeable skin damage from US transdermal drug delivery experiments (Miyazaki *et al.* 1992). One research group has examined the morphologic changes induced in *in vitro* hairless mouse skin and human skin after US exposure for transdermal drug delivery systems. The skins were immersed in a US water tank at 48 kHz and an intensity of 0.5 W/cm². Skins were compared with control skins under a scanning electron microscope and cells of the stratum corneum of the mouse skin surface were found to be almost completely removed. Also in the mouse skin, large craterlike pores with a diameter of 100 μm were formed sporadically in some of the skin samples. However, in human skin, the surface of skin exposed to US showed only slight removal of keratinocytes around the hair follicles. The researchers suggested that the removal of the stratum corneum and other alterations in hairless mouse and human skin may explain the enhancement of transdermal drug penetration (Yamashita *et al.* 1997).

In conclusion, results herein demonstrate a promising outcome for low-frequency US to be used for enhanced transport across the stratum corneum. These results indicate the feasibility of these transducers for transdermal drug delivery of insulin across human skin and that they have significant clinical potential.

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REFERENCES

- AIUM. Acoustic output labeling standard for diagnostic ultrasound equipment. Laurel, MD: American Institute of Ultrasound in Medicine, 1998.
- Asano J, Suisha F, Takada M, Kawasaki N, Miyazaki S. Effect of pulsed output ultrasound on the transdermal absorption of indomethacin from an ointment in rats. *Biol Pharm Bull* 1997;20:288–291.
- Bommannan D, Menon GK, Okuyama H, Elias PM, Guy RH. Sonophoresis. II. Examination of the mechanism(s) of ultrasound-enhanced transdermal drug delivery. *Pharm Res* 1992a;9:1043–1047.
- Bommannan D, Okuyama H, Stauffer P, Guy RH. Sonophoresis. I. The use of high-frequency ultrasound to enhance transdermal drug delivery. *Pharm Res* 1992b;9:559–564.
- Boucaud A, Tessier L, Machet L, Vaillant L, Patat F. Transdermal delivery of insulin using low frequency ultrasound. Proceedings of the IEEE 2000 Ultrasonics Symposium, San Juan, Puerto Rico, November 2000.
- Byl NN, McKenzie A, Halliday B, Wong T, O'Connell J. The effects of phonophoresis with corticosteroids: A controlled pilot study. *J Orthop Sports Phys Ther* 1993;18:590–600.
- Dogan A, Uchino K, Newnham RE. Composite piezoelectric transducer with truncated conical endcaps "cymbal." *IEEE Trans Ultrason Ferroelec Freq Control* 1997;44:597–605.
- Hippius M, Uhlemann C, Smolenski U, *et al.* In vitro investigations of drug release and penetration-enhancing effect of ultrasound on transmembrane transport of flufenamic acid. *Int J Clin Pharmacol Ther* 1998;36:107–111.
- Humulin® Information for the patient. Indianapolis, IN: Eli Lilly and Co, 1999.
- IEEE. Guide for medical ultrasound field parameter measurements. New York: IEEE, 1990.
- Johnson ME, Mitragotri S, Patel A, Blankschtein D, Langer R. Synergistic effects of chemical enhancers and therapeutic ultrasound on transdermal drug delivery. *J Pharm Sci* 1996;85:670–679.
- Krall LP. World book of diabetes in practice. New York: Elsevier, 1988.
- Levy D, Kost J, Meshulam Y, Langer R. Effect of ultrasound on transdermal drug delivery to rats and guinea pigs. *J Clin Invest* 1989;83:2074–2078.
- Mitragotri S, Kost J. Low-frequency sonophoresis: A noninvasive method of drug delivery and diagnostics. *Biotechnol Prog* 2000;16:488–492.
- Mitragotri S, Blankschtein D, Langer R. Ultrasound-mediated transdermal protein delivery. *Science* 1995;269:850–853.
- Mitragotri S, Blankschtein D, Langer R. Transdermal drug delivery using low-frequency sonophoresis. *Pharm Res* 1996;13:411–420.
- Mitragotri S, Blankschtein D, Langer R. An explanation for the variation of the sonophoretic transdermal transport enhancement from drug to drug. *J Pharm Sci* 1997;86:1190–1192.
- Miyazaki S, Mizuoka H, Kohata Y, Takada M. External control of drug release and penetration. VI. Enhancing effect of ultrasound on the transdermal absorption of indomethacin from an ointment in rats. *Chem Pharm Bull (Tokyo)* 1992;40:2826–2830.
- Montorsi F, Salonia A, Guazzoni G, *et al.* Transdermal electromotive multi-drug administration for Peyronie's disease: Preliminary results. *J Androl* 2000;21:85–90.
- Newnham RE, Dogan A. Metal-electroactive ceramic composite transducer. US Patent 5,1998;729:077, 1998.
- Newnham RE, Xu QC, Yoshikawa S. Transformed stress direction acoustic transducer. US Patent 4,999,819, 1991.
- Newnham RE, Xu QC, Yoshikawa S. Metal-electroactive ceramic composite actuators. US Patent 5,1994;276:657, 1994.
- Prausnitz MR. Reversible skin permeabilization for transdermal delivery of macromolecules. *Crit Rev Ther Drug Carrier Syst* 1997;14:455–483.
- Prausnitz MR. A practical assessment of transdermal drug delivery by skin electroporation. *Adv Drug Deliv Rev* 1999;35:61–76.
- Tachibana K. Transdermal delivery of insulin to alloxan-diabetic rabbits by ultrasound exposure. *Pharm Res* 1992;9:952–954.
- Tachibana K, Tachibana S. Transdermal delivery of insulin by ultrasonic vibration. *J Pharm Pharmacol* 1991;43:270–271.
- Tachibana K, Tachibana S. Application of ultrasound energy as a new drug delivery system. *Nippon Rinsho* 1998;56:584–588.
- Tressler JF, Cao W, Uchino K, Newnham RE. Finite element analysis of the cymbal-type flexensional transducer. *IEEE Trans Ultrason Ferroelec Freq Control* 1998;45:1363–1369.
- Wu J, Chappelow J, Yang J, Weimann L. Defects generated in human stratum corneum specimens by ultrasound. *Ultrasound Med Biol* 1998;24:705–710.
- Yamashita N, Tachibana K, Ogawa K, Tsujita N, Tomita A. Scanning electron microscopic evaluation of the skin surface after ultrasound exposure. *Anat Rec* 1997;247:455–461.
- Zhang I, Shung KK, Edwards DA. Hydrogels with enhanced mass transfer for transdermal drug delivery. *J Pharm Sci* 1996;85:1312–1316.