

Ultrasound Mediated Transdermal Transport of Insulin through *in vitro* Human Skin using Novel Transducer Designs

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Abstract

Recent studies have shown that ultrasound mediated transdermal drug delivery offers a promising potential for noninvasive drug administration. The purpose of this study was to improve low frequency (20 kHz) ultrasound methods for enhancing the transport of insulin across *in vitro* human skin. The feasibility of using ultrasound produced by small, lightweight novel transducers was explored for enhancing the transport of insulin across skin. Previous investigators have used ultrasound devices such as large, heavy sonicators or commercially obtained transducers for this type of research. These experiments carried out in this study used two low-profile novel ultrasound 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 $15.4 \pm 0.6 \text{ mW/cm}^2$ while 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 across *in vitro* human skin. Compared to passive transmission ($4.1 \pm 0.5 \text{ U}$) over an exposure period of one hour, the standard array facilitated over a seven-fold 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 four-fold increase in the ultrasound facilitated transmission compared to the control. These promising results indicate that low frequency ultrasound can be used in a practical device for enhanced transport across the *stratum corneum*.

Keywords: ultrasound, transdermal, drug delivery, insulin, skin

Introduction

Recent studies have shown that ultrasound mediated transdermal drug delivery offers promising potential for noninvasive drug administration (Mitragotri et al. 1995; Mitragotri et al. 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 ultrasound (also called sonophoresis or phonophoresis)) which are methods of enhancement (Prausnitz 1997; Prausnitz 1999; Montorsi et al. 2000). In the past, ultrasound has been used by physical therapists and physicians to treat patients with local musculoskeletal inflammation using topically applied steroids (Byl et al. 1993). More recently, the use of ultrasound is being explored for chemical activation of drugs by for treatment of cancers (sonodynamic therapy). For example, ultrasound energy can enhance effects of thrombolytic agents such as urokinase (Tachibana and Tachibana 1998).

The focus of this research is to develop a practicable method for drug delivery using low frequency (20 kHz) ultrasound to enhance the transport of insulin across skin using novel transducer designs. Of note, high frequency ultrasound in the MHz range has been used for transdermal delivery of various proteins and drug. Previous attempts to use high frequency ultrasound (≈ 1 MHz and $\approx 1-3$ W/cm²) to enhance transdermal drug delivery have produced inconsistent results and were found to vary significantly from drug to drug (Bommannan et al. 1992a; Bommannan et al. 1992b; Mitragotri et al. 1997). Pulsed ultrasound at 1 MHz has been used to increase transdermal absorption of indomethacin from an ointment in rats (Asano et al. 1997). Also, the combination of

chemical enhancers and therapeutic ultrasound (1 MHz, 1.4 W/cm², 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 ultrasound. Ultrasound 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 (1-3 MHz) and low (\approx 20 kHz) frequency ultrasound appears to be that low frequency ultrasound enhances transdermal drug transport 1000 times greater than high frequency ultrasound (Mitragotri et al. 1996). The hypothesis for the physical mechanism is that low-frequency ultrasound enhances transdermal transport through aqueous channels in the *stratum corneum* which is generated by the distortion of the lipid bilayer due to cavitation.

However, the physical mechanism of the enhancement using ultrasound is far from being fully understood. Some researchers have concluded that at 168 kHz using CW ultrasound and at 1.9×10^5 Pa 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 the unenhanced transdermal drug delivery (Wu et al. 1998).

Transport of both vasopressin and insulin across *in vitro* human skin has been demonstrated using a 20 kHz sonicator (Sonicator W385, Heat Systems, Farmingdale, NY) over a period of 5 hours 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 ultrasound sonicator operating at intensities from 12.5 - 225 mW/cm² (Mitragotri et al. 1995).

The purpose of this research is to explore the feasibility of using ultrasound produced by novel transducers for enhancing the transport of insulin across *in vitro* skin. 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 was determined. As shown above, much of the drug delivery literature reports an intensity values but without indicating essential information such as if it was the spatial peak temporal peak or temporal average (IEEE 1990). The goal of this research is to introduce a practicable (i.e. low-cost, light weight) ultrasound device to noninvasively transport insulin, a large molecular weight protein (MW = 5807.69 Da), across *in vitro* skin. To accomplish this task, this research will explore 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 ultrasound 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; Newnham et al. 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 20-50 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 allow 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 the cymbal transducer. A two-by-two elemental pattern was used for the four transducers; the final array was in a 37 x 37 x 7 mm³ block (Fig. 2)

The block diagram for the driving equipment for the cymbal and array transducers is shown in Fig. 3. The radio frequency (RF) signal driving a 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 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 capacitors (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 1V (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.

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 9mm diameter, calibrated (-212.25 dB re 1 V/ μ Pa) miniature omnidirectional reference hydrophone (Model TC4013, S/N: 5199093, RESON, Inc., Goleta, CA) in a 51 x 54 x 122 mm³ partially anechoic tank containing degassed, distilled water. A computer-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 area was 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}) was determined over a plane 1 mm from each transducer face using the hydrophone based on

3-5 scanings of each transducer for a mean and standard deviation of the results (IEEE 1990; AIUM 1998).

Skin handling

Whole *in vitro* human skin was 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, sex, race, skin location and cause of death were documented. Human skin from the abdomen was used for determining enhanced transport of insulin using ultrasound.

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 the skin was limited to 31 mm by two Plexiglas[®] rings which 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.

Spectrophotometer calibration

Absorbance measurements using a spectrophotometer (Model UV VIS 160M, Shimadzu, Columbia, MD) were used for determining the enhancement of insulin delivery across skin using ultrasound. Ultrasound 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 Units/ml in a 10 ml bottle (Eli Lilly and Co 1999).

Insulin and saline (10 mM phosphate buffered saline-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 20-30 mins before transfer to a quartz cuvette with a nominal volume of 1.4 ml and 10 mm path length

(Model 9BQ10, Starna 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 versus the insulin (U/ml) for each wavelength. Regression analysis of the absorbance based on insulin concentrations generated a linear regression equation along with the R^2 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 are significantly greater than chance. For all the data presented a double asterisk was used if the F value is less than the 0.01 level of significance.

Insulin transmission through in vitro skin

For maintaining constant humidity and temperatures, experiments were performed in a custom built 61 x 101 x 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 prior to an experiment the phosphate buffered saline (Catalog No. P-3813, SIGMA, St. Louis, MO) 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. Once the receiver compartment was filled with saline the compartments were clamped together. Care was taken to remove all bubbles from the receiver and insure 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 one hour of either a control or ultrasound 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 standard deviation ($\bar{x} \pm \text{s.d.}$) at each wavelength was 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 one hour 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 1V_{pp} signal with a 20% duty cycle amplified by 50 dB

with a 1 s pulse repetition period. Each device was scanned 3-5 times to produce a mean and standard deviation of the intensity results. Table 1 lists the mean and standard deviation ($x \pm \text{s.d.}$) 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 to the single element cymbal transducer and the stack array under similar driving settings.

Insulin transmission through in vitro skin

In vitro human skin samples from the abdominal region were used for determining enhanced transport of insulin by ultrasound using either the single element cymbal transducer, stack array or standard array. Spectrophotometer calibration results for the absorbance versus Humulin[®]R or Humalog[®] insulin concentration (U/ml) produced a linear regression with an R^2 no less than 0.985. After exposure to either a control or ultrasound setting for one hour, samples from the receiver compartment were placed in the spectrophotometer and the absorbance was recorded. Using the calibration curve, the amount of insulin transported across the skin over a period of one hour was determined. Table 2 lists the experimental results of the insulin transport under the control and ultrasound conditions using three transducers. Data are listed as its mean and standard deviation ($x \pm \text{s.d.}$) along with the number of experimental trials (n value) for each skin sample. Compared to the control experiment, an ANOVA found the use of ultrasound for transdermal transport of insulin, whether it was with the single element cymbal transducer, stack array or standard array, to be statistically significant.

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 ultrasound 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 ultrasound (Mitragotri et al. 1995). Other researchers have investigated the *in vitro* penetration and the *in vivo* transport of flufenamic acid in skin with ultrasound (Hippius et al. 1998). In the flufenamic acid study, ultrasound exposure was from 5-30 min with intensities up to 1.5 W/cm². Although there was a pronounced effect of ultrasound on the transmembrane absorption of the drug, there was also a rise of temperature up to 4.5 °C. Ultrasound at 1 MHz has also been used to enhance the transdermal absorption of indomethacin studied in rats using intensities from 0.25 - 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 ultrasound increased beyond 1 W/cm² (Miyazaki et al. 1992).

The goal of this research was to determine if an ultrasound 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 *in vitro* and *in vivo* skin using intensities as low as 12.5 mW/cm² (Mitragotri et al. 1995). Based on the spatial peak temporal peak intensity results, the standard array produced intensities which 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 sonicators can weigh almost a kilogram or more while the standard array weighs less than 22 grams. 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 ultrasound compared to passive transmission (i.e. control conditions - no ultrasound). Over a one hour period using the single element cymbal transducer with Humulin[®]R, almost twice the amount of insulin was transported across the skin via ultrasound (7.4 U) compared to the control (4.1 U). With Humulin[®]R, use of the stack (20.3 U) and standard (45.9 U) ultrasound array results in a four- or seven-fold increase, respectively. Although the passive transmission (control, 7.0 U) across the skin of Humalog[®] was slightly higher than Humulin[®]R, ultrasound again increased the transmission of the insulin. Based the Humulin[®]R single element results along with the expense of human skin, Humalog[®] experiments using the single element was 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) while the standard array had a greater effect on the 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 significant at p-value of 0.05 or better.

Although not presented here as a formal study, preliminary tests have been performed on the post sonicated 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 breakdown 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 three times each day for a total of 36 U per day (Krall 1988). Based on these results, the standard array can deliver 30-45 U per hour across *in vitro* human skin; therefore one dose can be delivered in less than 30 mins 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) (Tachibana 1992; Mitragotri et al. 1995; Zhang et al. 1996; Boucaud et al. 2000) (Table 1). Over a frequency range of 20-105 kHz, enhanced transport in the presence of ultrasound has been shown in both *in vitro* and *in vivo* experiments. Yet all of the experiments were performed using either an ultrasound sonicator, ultrasonic bath or commercial transducer. The major drawback so far in exploiting ultrasound for noninvasive drug delivery has been the large size and poor mobility of sonicator.

Visual and microscopic examination of the ultrasound exposed skin did not reveal any noticeable damage to the skin. Other researchers have reported noticeable skin damage from ultrasound transdermal drug delivery experiments (Miyazaki et al. 1992). One research group has examined the morphological changes induced in *in vitro* hairless mouse skin and human skin after ultrasound exposure for a transdermal drug delivery systems. The skins were immersed in an ultrasound water tank at 48 kHz and an

intensity of 0.5 W/cm^2 . Skins were compared to control skins under a scanning electron microscope and found cells of the stratum corneum of the mouse skin surface were almost completely removed. Also in the mouse skin, large craterlike pores with a diameter of 100 microns were formed sporadically in some of the skin samples. However in human skin, the surface of skin exposed to ultrasound 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 ultrasound to be used for enhanced transport across the *stratum corneum*. These results indicate that the feasibility of these transducers for transdermal drug delivery of insulin across human skin and have significant clinical potential.

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Reference List

- 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* 1992; 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* 1992; 9:559-564.
- Boucaud A, Tessier L, Machet L, Vaillant L, and Patat F. Transdermal delivery of insulin using low frequency ultrasound. Proceedings of the IEEE 2000 Ultrasonics Symposium, San Juan Porto 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 Nov; 18:590-600.
- Dogan A, Uchino K, Newnham RE. Composite piezoelectric transducer with truncated conical endcaps "cymbal". *IEEE Trans Ultrason , Ferroelect , Freq Contr* 1997; 44:597-605.

Eli Lilly and Co. 1999 Aug 13. Information for the patient Humulin® Regular Insulin.
Indianapolis, IN: Eli Lilly and Company. PA 6326.

Hippius M, Uhlemann C, Smolenski U, Schreiber U, Reissig S, Hoffmann A. 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.

IEEE Guide for Medical Ultrasound Field Parameter Measurements. New York: Institute of Electrical and Electronics Engineers, Inc. 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*. Elsevier. New York NY, 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, 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.
- Mitragotri S, Kost J. Low-frequency sonophoresis: a noninvasive method of drug delivery and diagnostics. *Biotechnol Prog* 2000; 16:488-492.
- 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, Barbieri L, Colombo R, Brausi M, Scattoni V, Rigatti P, Pizzini G. Transdermal electromotive multi-drug administration for Peyronie's disease: preliminary results. *J Androl* 2000; 21:85-90.
- Newnham RE, Dogan A, inventors. 1998 Mar 17. Metal-electroactive ceramic composite transducer. U.S. Patent 5,729,077.
- Newnham RE, Xu QC, Yoshikawa S, inventors. 1991 Mar 12. Transformed stress direction acoustic transducer. U.S. Patent 4,999,819.
- Newnham RE, Xu QC, Yoshikawa S, inventors. 1994 Jan 4. Metal-electroactive ceramic composite actuators. U.S. Patent 5,276,657.
- 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 , Ferroelect , Freq Contr* 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.

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

Intensity (mW/cm²)	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

Table 2. A total of 36 abdominal *in vitro* skin samples were used for 23 Humulin[®]R and 13 Humalog[®] insulin transmission experiments. The results indicate the units (U) of insulin delivered over an exposure period of one hour.

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)

* F value exceeds the 0.05 level of significance.

** F value exceeds the 0.01 level of significance.

Figure Legends

Fig. 1.

(a) The single element transducer, designated the "cymbal" transducer, was made of piezoelectric material PZT-4 (lead zirconate-titanate) and operated at a frequency of 20 kHz. The cymbal consists of a piezoelectric disk placed between two titanium caps with air cavities beneath the caps which gives rise to radial oscillations of the disk.

(b) View of a 12 mm diameter cymbal transducer (right). For experiments, the cymbal transducer was connected with a coaxial cable and housed in polymer material in order to ensure electrical insulation between the end caps (center). The transducer was encased in a housing for stability and positioning in water (left).

Fig. 2.

For the standard array made up of four cymbal transducers, the cymbal elements were connected in parallel, encased in URALITE[®] polymer and arranged in a two-by-two elemental pattern. The dimensions of the array were 37 x 37 x 7 mm³.

Fig. 3.

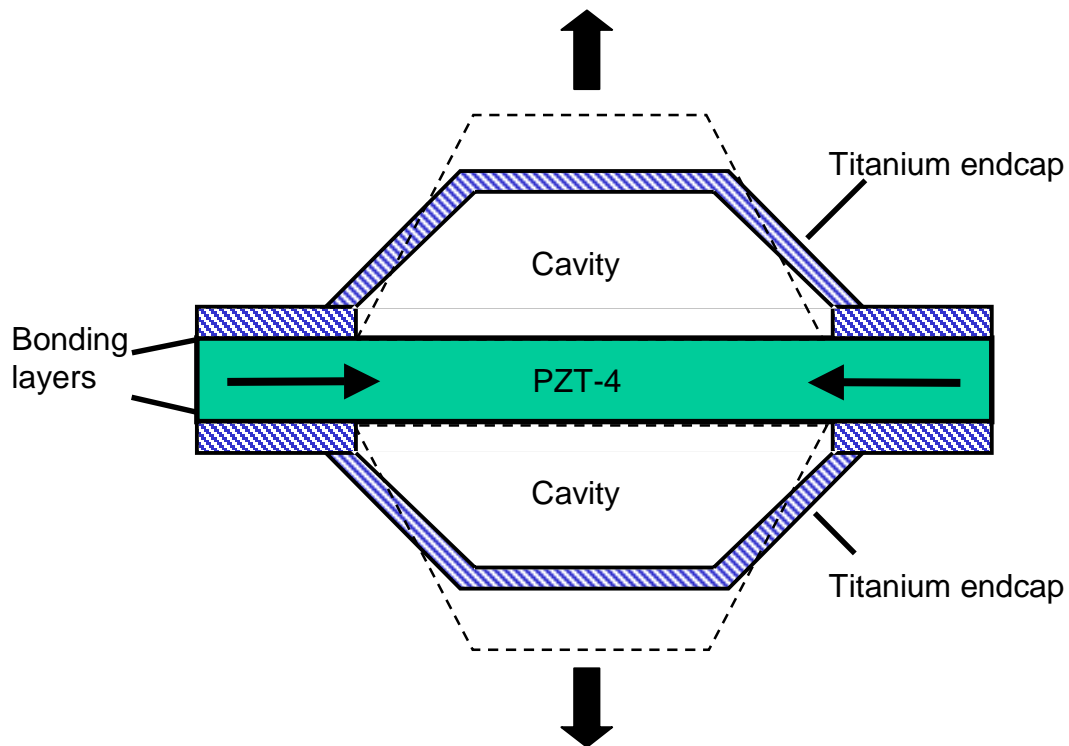
Experimental set-up of the function generator and the amplifier used for driving the cymbal single element transducer and four element array. For the experiments the three transducers were electrically driven under similar conditions. The pulsed signal ($f = 20$ kHz) from the signal generator was at 1V_{pp} with a pulse duration of 200 ms and pulse repetition period of 1 second (i.e. 20% duty cycle). Amplifier gain was 50 dB. Intensity results in mW/cm² were determined over a plane 1 mm from the transducer face.

Fig. 4.

- (a) The Franz diffusion cell was designed and specially fabricated for this project (figure not to scale). The cell consisted of a upper donor compartment and lower receiver compartment (100 ml) . The transmission experiments placed the skin surface 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.
- (b) Photograph of the Franz diffusion cell.

Figure 1

(a)



(b)

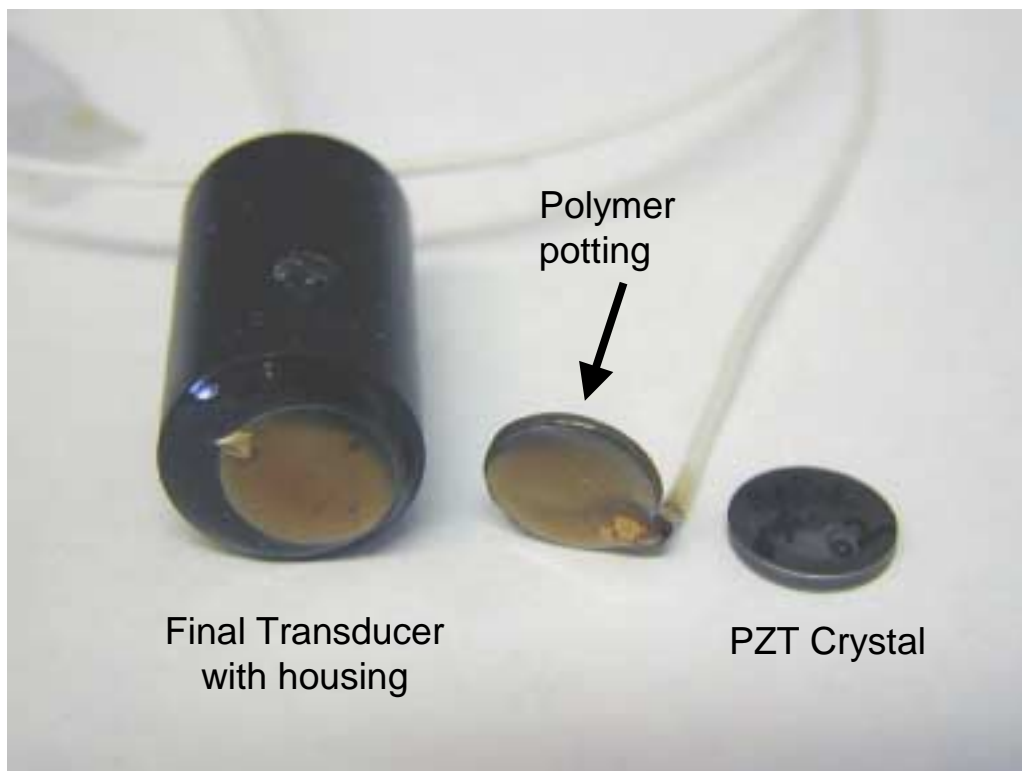


Figure 2

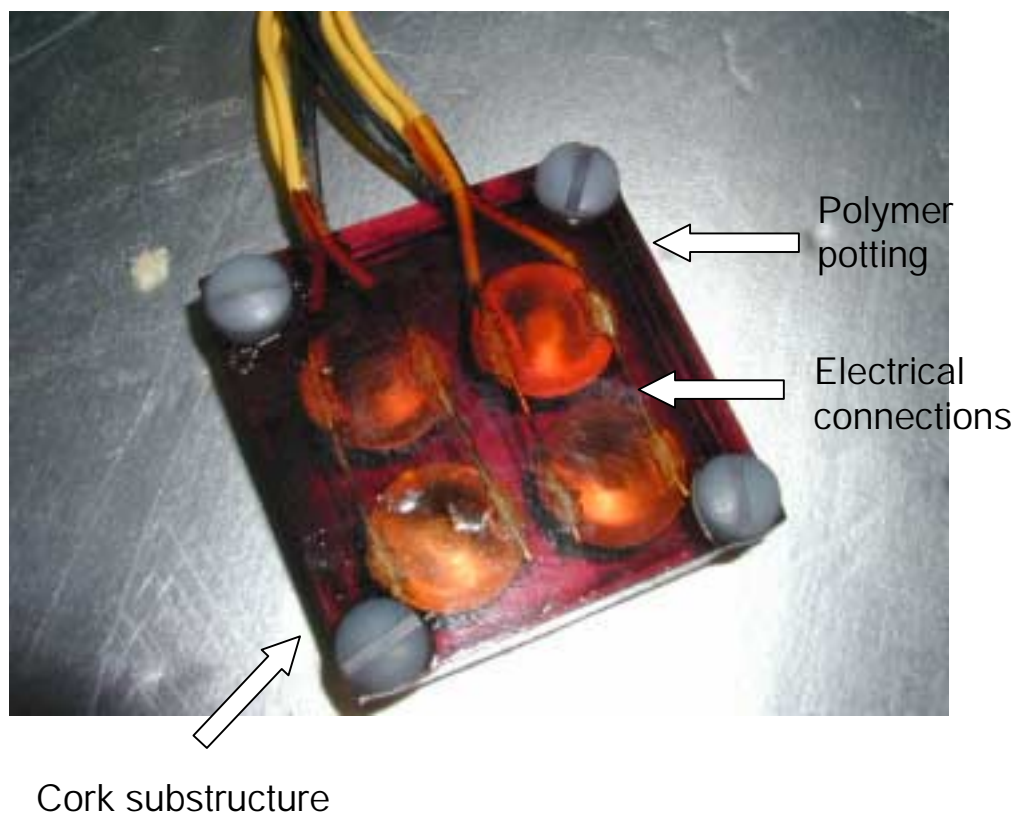


Figure 3

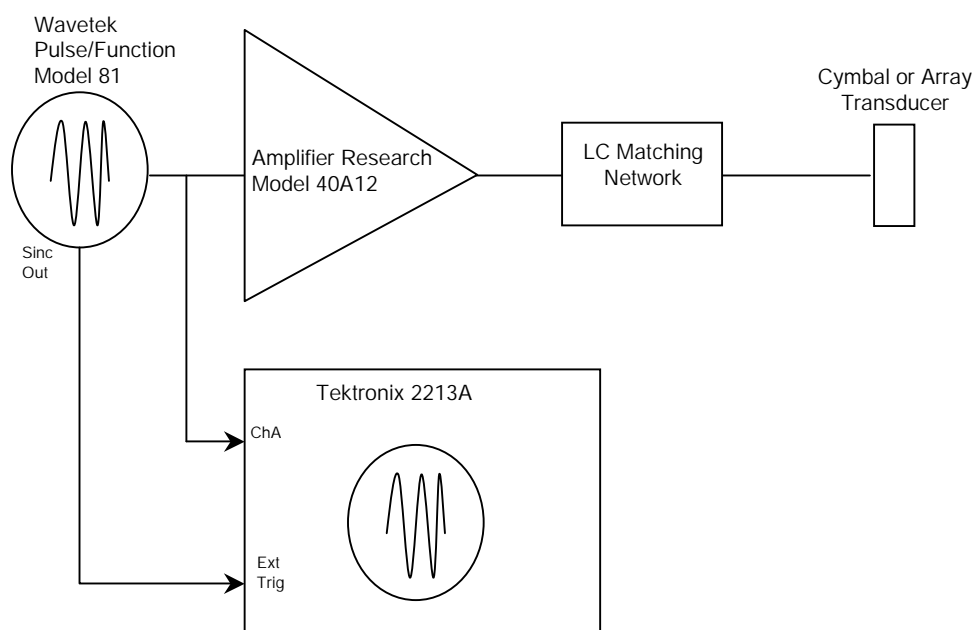
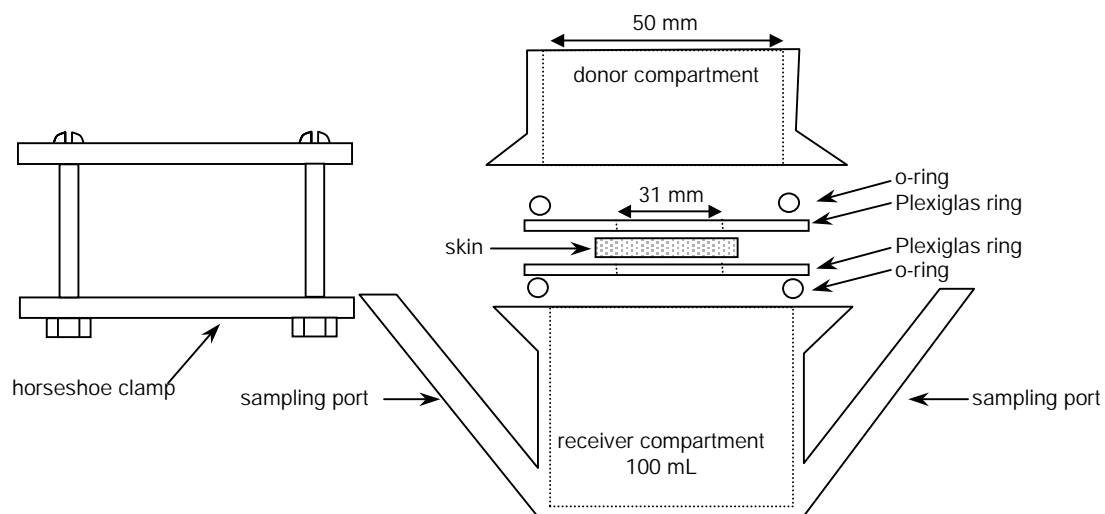


Figure 4



(a)



(b)