Research Paper

Ultrasound Mediated Transdermal Insulin Delivery in Pigs Using a Lightweight Transducer

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Purpose. In previous studies, ultrasound mediated transdermal drug delivery has shown a promising potential as a method for noninvasive drug administration. For prospective future human application, this study was designed to determine the feasibility of lightweight cymbal transducer array as a practical device for noninvasive transdermal insulin delivery in large pigs.

Materials and Methods. Six Yorkshire pigs (100–140 lbs) were divided into two groups. As the control ($n=3$), the first group did not receive any ultrasound exposure with the insulin. The second group ($n=3$) was treated with ultrasound and insulin at 20 kHz with an $I_{sptp}=100$ mW/cm^2 at a 20% duty cycle for 60 min. With the pigs in lateral recumbency after anesthesia, the ultrasound transducer with insulin was placed on the axillary area of the pig. At the beginning and every 15 min up to 90 min, the blood glucose level was determined using a glucose monitoring system. To compare the results of individual animals, the change of blood glucose level was normalized to each animal's initial glucose value at the start of the experiment.

Results. Although each animal had a different initial glucose level, the mean and standard error for the six animals was 146±13 mg/dl. For the control group, the blood glucose level increased to 31±21 mg/dl compared to the initial baseline over the 90 min experiment. However for the ultrasound with insulin treated group, the glucose level decreased to −72±5 mg/dl at 60 min ($p < 0.05$) and continued to decrease to −91±23 mg/dl in 90 min ($p<0.05$).

Conclusion. The results indicate the feasibility of ultrasound mediated transdermal insulin delivery using the cymbal transducer array in animal with a similar size and weight to a human. Based on these result, the cymbal array has potential as a practical ultrasound system for noninvasive transdermal insulin delivery for diabetes management.

KEY WORDS: diabetes; drug delivery; insulin; transducer; ultrasound.

INTRODUCTION

Use of needles for multiple injection of drugs, such as insulin for diabetes, can be painful. As a result, prescribed drug noncompliance can results in severe medical complications. Several noninvasive methods exist for transdermal drug delivery. These include chemical mediation using liposomes and chemical enhancers or physical mechanisms such as microneedles, iontophoresis, electroporation, and ultrasound (1–5). Ultrasound enhanced transdermal drug delivery presents advantages over traditional injection drug delivery methods which are invasive and painful. Currently only a small amount of drugs have been successfully administered transdermally for clinical applications because of the low skin permeability to these relatively large molecules. This low permeability is mainly attributed to the outermost skin layer, *stratum corneum*, which consists of a condensed and ordered structure of cells, keratinocytes, compassed by lipid bilayers. Once the drug crosses stratum corneum, the next epidermal layer is less problematic to traverse, and consequently the drug can reach the capillary bed to be absorbed (6,7).

Recent reviews have shown that ultrasound mediated transdermal drug delivery offers promising potential for noninvasive drug administration (8–10). The working principle of sonophoresis, although not completely understood, has been suggested to be the result of cavitation (11–14). Low frequency ultrasound is capable of generating microbubbles in the water and tissue. These bubbles allow water channels to be produced within the lipid bilayers. The resulting disorder created in the stratum corneum facilitates the crossing of a hydrophilic drug or molecule. To this end, the number of drugs and compounds which have been shown to transdermally cross skin via ultrasound is ever increasing (15).

Noninvasive methods for transdermal delivery of insulin have particular public interest due to the increasing problem of diabetes. Approximately 16 million people suffer from

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diabetes mellitus in the United State alone. From a human and economic perspective, it is one of the most costly diseases and management of diabetes often requires painful repetitive insulin injections up to three to four times each day (16,17). Thus the research for safe and convenient noninvasive insulin delivery is increasing every year. 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 (6,18–23). Many early experiments were performed using either an ultrasound sonicator, ultrasonic bath or commercial transducer. For example investigators have demonstrated effective *in vivo* transport of insulin at 48 kHz using an ultrasonic bath (24) and 105 kHz (18) using a commercially obtained transducer. The major drawback so far in exploiting ultrasound for noninvasive drug delivery is the large size and poor mobility of the ultrasound device. Commercial sonicators are large, heavy, table-top devices specifically designed for lysis of cells, catalyzing reactions, creating emulsions or cleaning.

A recent comprehensive review on ultrasound drug delivery states that "small-sized low-frequency transducers need to be developed so that patients can wear them" (9). There are several possible low frequency transducer designs that can be used in a drug delivery application, such as the low frequency flextensional resonators (25), tonpilz transducers (26), or "thickness"-type resonators (27). One other inexpensive candidate is the low-profile, light weight cymbal transducer for a portable device. This flextensional transducer has a thickness of less than 2 mm, weighs less than 3 g and resonates between 1 and 100 kHz depending on geometry (28–30). Multiple cymbals can be patterned side-by-side into 2×2 and 3×3 arrays for increased spatial intensity fields for drug transport. Consequently the use of the low-profile, light weight cymbal ultrasound array has previously demonstrated transport enhancement of insulin across *in vitro* human skin (23), *in vivo* rats (31,32) and rabbits (33,34). Additionally the cymbal design has also been used for noninvasive glucose sensing (35).

Yet for prospective human application, a milestone to a practical clinical device of this research requires that the efficacy of the cymbal array be demonstrated on animals larger than rats and rabbits. Larger and possessing a blood volume similar to humans, pigs are an appropriate animal models for further experimentation in evaluating the practicability of this ultrasound device. Based on the positive results from our previous research, the purpose of this study was to determine the feasibility of ultrasound mediated transdermal delivery of insulin *in vivo* using pigs to mimic possible future applications in humans.

MATERIALS AND METHODS

Ultrasound Transducer Array

Details regarding the design and construction of the cymbal transducer and the multi-element array have been described elsewhere (28–30). Briefly, the cymbal transducer is a novel flextensional transducer capable of producing very low frequencies (Fig. 1a). The cymbal transducer has a compact, lightweight structure with an adjustable resonance
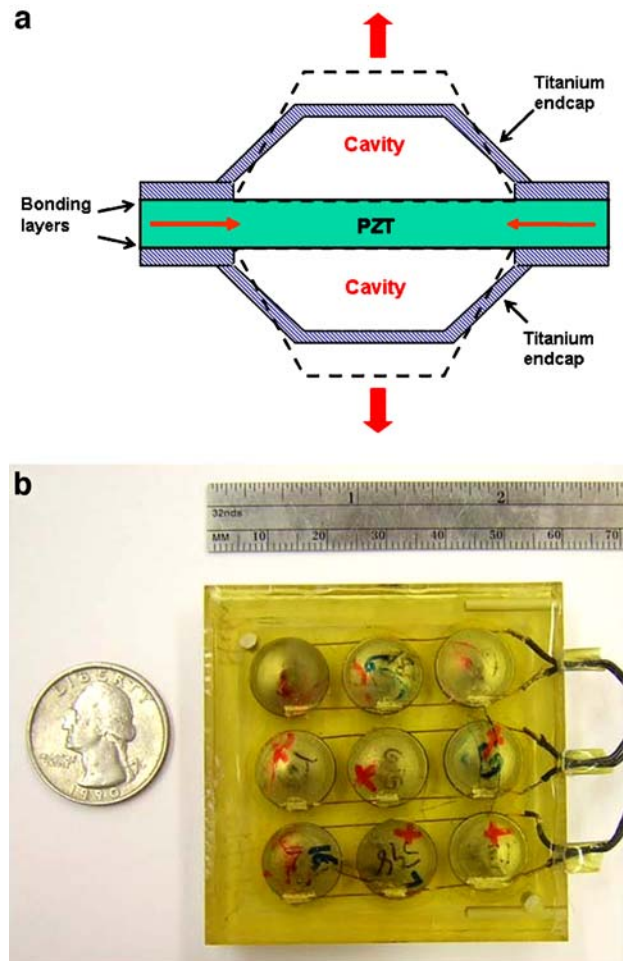

Fig. 1. a The cymbal transducer operated at a frequency of 20 kHz. Placed between two titanium caps with air cavities beneath, the motion of the cymbal disk moves the caps which to give rise to radial oscillations. *Dashed lines* represent the flexing of the caps and the *arrows* indicate the motion. **b** A light weight, low-profile array was constructed using nine cymbal transducers which were connected in parallel and encased in a polymer.

frequency. In the cymbal transducer design, the caps on the lead zirconate-titanate (PZT) ceramic contained a shallow cavity beneath its inner surface. The fundamental mode of vibration is the flexing of the end caps caused by the radial motion of the ceramic represented by the dashed lines in Fig. 1a. Therefore, the overall displacement of the device is a combination of the axial motion of the disk plus the radial motion amplified by the end caps. Amplification factors can be as high as 40 times that of the ceramic by itself (36). Specifically, the piezoelectric disc was made from PZT-4 (Piezokinetics, Inc., Bellefonte, PA), had a diameter of 12.7 mm, and was 1 mm thick. PZT-4 was chosen because this material has a high failure voltage threshold compared to ceramics with similar efficiency. Caps were made of 0.25-mm thick titanium while the thin glue layer between the caps and the ceramic disk was made of Eccobond® (Emerson & Cuming, Billerica, MA) epoxy. For the array, four transducers were connected in parallel and encased in URALITE® polymer (FH 3550, H.B. Fuller, St. Paul, MN, USA) to produce a transducer array arrangement. A three-by-three

(3×3) elemental pattern was used for the array and was in a 56×56×8 mm³ block (Fig. 1b).

To drive the array, a radio frequency (RF) signal was generated by a frequency pulse/function generator (Agilent 32250A, Palo Alto, CA) and amplified by an RF amplifier (Model 40A12, Amplifier Research, Souderton, PA). The electrical impedance of the array was tuned to the output impedance of the amplifier by an external inductor-capacitor tuning network. 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 experiments, the signal generator operated at 20 kHz 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. Pulsed ultrasound was used to avoid damaging heat build-up to either the array or animal's skin.

Ultrasound Exposimetry

The intensity was determined according to exposimetry guidelines established by the American Institute of Ultrasound in Medicine (37,38). For the acoustic field at a plane 1 mm from the transducer face, the ultrasonic intensities from the array were measured with a calibrated miniature (4 mm diameter) omnidirectional reference hydrophone (Model TC4013, S/N: 5199093, RESON, Inc., Goleta, CA). The cymbal array was submerged in a water tank (51×54×122 cm³) which was made almost anechoic by placing 1.27 cm thick rubber sound absorbing material around its wall. A custom made degasser, built in-house, reduced the dissolved oxygen content of the distilled water to 1–2 ppm to reduce cavitation effects. Pulse period, duty cycle and exposure time of the signal from the frequency generator and hydrophone was acquired using an Agilent 54622A 100 MHz digitizing oscilloscope (Agilent, Palo Alto, CA).

Precise, computer controlled positioning of the hydrophone was performed by a Velmex Positioning System (Velmex Inc., East Bloomfield, NY). Pressure waves detected by the hydrophone were recorded by a digitizing oscilloscope. A computer-controlled exposimetry positioning system was used for automated scanning. The scanning step size for each device was 1 mm and the scanning area was 80×80 mm². Spatial peak-temporal peak (I_{sptp}) and spatial average-temporal peak (I_{satp}) intensities were determined over a plane 1 mm from the array face using the hydrophone based on 3–5 scanning of the array for a mean and standard deviation of the intensity results. The intensity of cymbal transducer array was I_{sptp}=100.8±0.6 mW/cm² and I_{satp}=30.7±0.6 mW/cm².

Animal Experiments

The pigs were anesthetized and euthanatized by procedures approved by the Institutional Animal Care and Use Committee (IACUC) at the Pennsylvania State University. Six Yorkshire pigs (100–140 lbs) obtained from the Penn State Swine Center were divided into two experimental groups. As the control (n=3), the first group did not receive any ultrasound exposure with the insulin while second group (n=3) was treated with ultrasound and insulin. Each animal was pre-anesthetized for intubation with a combination of ketamine hydrochloride (10–12 mg/kg intramuscularly,

Ketaject®, Phoenix, St. Joseph, MO) and sodium xylazine (1–2 mg/kg intramuscularly, Xyla-Ject®, Phoenix, St. Joseph, MO). Pigs were fitted with an intravenous (IV) catheter in the auricular vein and an endotracial tube (size 6–7) was inserted into the airway. Anesthesia throughout the remaining experiment was maintained to surgical depth via inhalant isoflurane (Isothesia™, Abbott Laboratories, North Chicago, IL) using an inhalational anesthesia unit (Narkovet Deluxe, North American Drager, Telford, PA).

The axillary area of the pigs were shaved using an electric shaver, and a depilatory agent was applied to the skin of both groups, control and exposure, to eliminate any remaining hair. With the pig in the lateral recumbency (Fig. 2a), a 1 mm thick, water-tight standoff was attached (Fig. 2b) between the skin and the array using tissue glue (Vetbond®, 3M, St. Paul, MN). The reservoir within standoff was filled with insulin (Humulin® R, rDNA U-100, Eli Lilly and Co., Indianapolis, IN) through a small hole in the back of the array for both the control and exposure experiments. To prevent disruption of ultrasound transmission, care was taken to remove all bubbles from the solution in the reservoir (standoff) between the axillary area and the array.

At the beginning of the experiment, blood sample (0.3 ml) was collected from the ear vein of each pig for a baseline glucose level analysis. The glucose level (mg/dl) in the blood was determined using ACCU-CHEK™ blood glucose monitoring system (Roche Diagnostics Co., Indianapolis, IN, USA). Multiple blood samples (2–4 each time) were taken
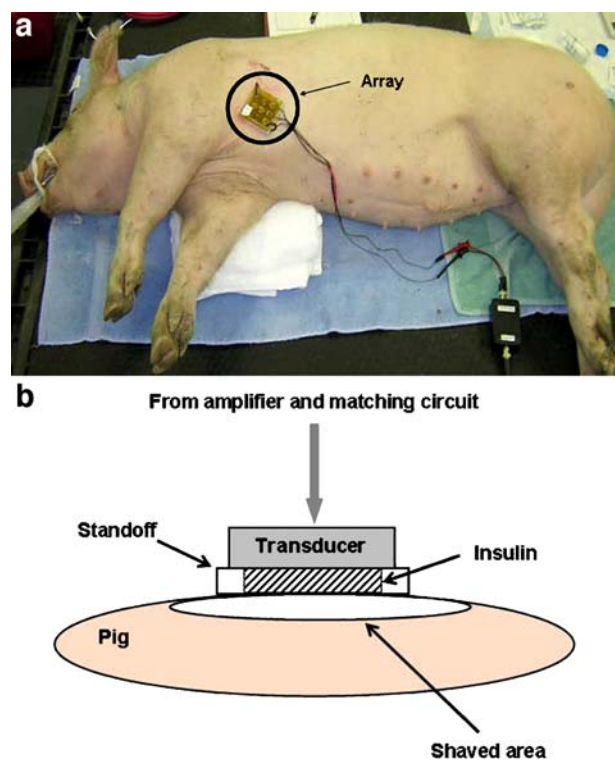

Fig. 2. a Photograph of a transdermal insulin delivery experiment with pig placed in a lateral recumbent position with the array attached. **b** Illustration of the experiment with a 1 mm thick water tight standoff arranged between the axillary area and the array. The reservoir within the standoff was filled with insulin through a small hole in the back of the array.

every 15 min for 90 min. Elapsed time from the initial injection of the ketamine-xylazine until the first glucose measurement was no greater than 15 min. For comparison between the pigs, the change in the blood glucose level was normalized to a baseline with respect to each animal's initial blood glucose recording at 0 min.

For comparison between the results of each pig, the change of blood glucose level was normalized with respect to the initial blood glucose level of each pig. The control group (three pigs) used insulin inside the reservoir without ultrasound exposure while the second group (three pigs) was treated with ultrasound and insulin at 20 kHz with an $I_{sptp} = 100$ mW/cm^2 for 60 min. For both groups, the standoff reservoir with the insulin or saline was removed at 60 min although glucose determination continued until 90 min from the start. At the end of the experiments, the pig was euthanized (Pentobarbitol, Fatal Plus®, 130 mg/kg IV, Vortech Pharmaceuticals, Ltd., Dearborn MI) under anesthesia.

Statistical analysis was performed using Microsoft Excel® (Microsoft Corp., Redmond, WA). The blood glucose verses time data were pooled for each group and analyzed as its mean and standard error (x ± s.e.). An ANOVA was used to analyze the statistical significance of the differences among the means of groups. The p-value was used to determine if the between-group differences are significantly greater than chance. For all the data, a single or double asterisk was used if the p-value is less than the 0.05 or 0.01 level of significance, respectively.

RESULTS

Results of the ultrasonic transdermal insulin delivery in large pigs for the two groups are graphed (Fig. 3) as the change in the blood glucose level during the 90 min experiment in terms of the mean and standard error. After the pigs were anesthetized, the average initial glucose level of the six pigs was 146 ± 13 mg/dl. Generally for pigs, the blood glucose level is approximately 100–110 mg/dl (39,40). As mentioned, for comparison between the pigs the change in the blood glucose level was normalized to a baseline which was the initial glucose level for each pig.

For the control group (insulin without ultrasound), the glucose level increased to 31 ± 21 mg/dl compared to the initial baseline over the 90 min experiment. The slope of this increase was +20 mg/dl/h ($r^2 = 0.9$). In contrast with the ultrasound exposure group (insulin with ultrasound), the glucose level decreased to −74 ± 5 mg/dl at 60 min and continued to decrease to −91 ± 9 mg/dl at 90 min after the stand off was removed. Additionally, a gross examination of the pig's skin was performed after exposure to look for visible lesions on the skin surface. Visual examination of the post ultrasound exposed skin did not indicate any noticeable damage or significant change to the skin.

To determine the statistical significance between the results in Fig. 3 of the two groups at the 15 min increment time points of experiment, an ANOVA was used to analyze this data. Asterisks above a glucose-time data point were used if there existed statistical difference between the control and exposure. At 15 min the analysis showed no statistical difference between the groups. However comparison of the control against the ultrasound exposure groups at 30 min and
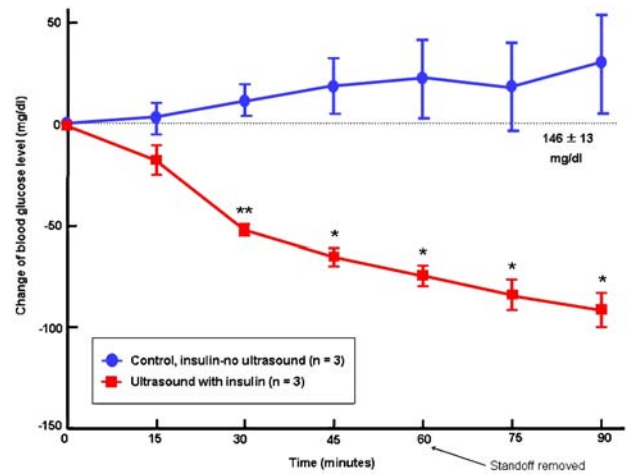

Fig. 3. Over a period of 90 min, the blood glucose level of pig decreased to −91±9 mg/dl at 90 min for ultrasound mediated transdermal insulin delivery. An ANOVA analysis of the control and ultrasound exposed groups at 30 min and greater indicated that the results were statistically significant at p values of 0.05 or better.

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DISCUSSION

The goal of this research was to determine if a lightweight, low-profile ultrasound device based cymbal transducer device could be used for *in vivo* transdermal insulin in animals which approximate the size and weight of a human. Although commercial sonicators have been admirable devices for demonstrating drug delivery, the ultrasonic probe or converter from a sonicators can weigh almost a kilogram or more while the cymbal array weighs less than 38 g. Individual cymbals (Fig. 1a) can be arranged into multi-element array designs (Fig. 1b) since this can increase the effective aperture of ultrasound area with respect to skin area and some research indicates that the delivery dose increases with ultrasound exposure area (23). Interestingly the cymbal design originates from underwater research for Navel applications and current research is underway to incorporate existing battery technology in the miniaturization of portable power (41).

Generally xylazine causes hyperglycemia in rats and rabbits (42–45). Specifically for pigs, xylazine suppresses insulin release (46) which resulted in the higher initial average blood glucose level of 146 mg/dl pigs. The mean value for normal blood glucose levels for pigs is approximately 108.5 mg/dl (40) yet the use of the xylazine appears to have the continual effect of increasing the glucose level at a rate of 20 mg/dl/h even after the experiment started. This may indicate either the lack of passive permeability of the pig's skin to the insulin or the sustained consequence of the xylazine. Yet the useful effect of using these insulin suppressed pigs was to demonstrate the feasibility of reducing a high glucose level (≈150 mg/dl) to a normal glucose level (below 100) albeit for a pig model. In contrast to the control group, the ultrasound with insulin group showed a blood glucose level decrease of −91 mg/dl at 90 min compared to the baseline. Moreover compared to the control at 90 min, the

difference between the two groups was approximately 120 mg/dl and the ANOVA analysis indicated there was a statistical difference ($p>0.05$) between the two groups at the time points of 30 min and greater.

To examine these results in terms of human diabetes, a person is considered diabetic if their blood sugar level is above 140 mg/dl after fasting. People without diabetes have fasting sugar levels that generally run between 70–110 mg/dl. When fasting, glucose of 110–126 mg/dl is classified as impaired fasting glucose, 140–200 mg/dl is impaired glucose tolerance and greater than 200 mg/dl is considered diabetic (47–49). In this case a diabetic person would need to inject enough insulin to reduce their blood glucose by about 100 mg/dl. Summarizing our previous ultrasound exposure results using hyperglycemic animals at 90 min, the glucose level continued to decrease to -296.7 ± 52.8 mg/dl in rats and -208.1 ± 29 mg/dl in rabbits (23,33). Although the decrease in blood glucose in this pig research was not as large in the rats and rabbits, this may be due to the fact that the blood volume and body mass is far greater for pigs. Nevertheless the results indicate that the array was capable of safely reducing a diabetic glucose level to a normal range. In conclusion, results herein demonstrate a promising pre-clinical outcome for the low profile cymbal array to be used for ultrasound enhanced *in vivo* insulin transport using a an animal model which mimics human use.

ACKNOWLEDGEMENTS

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