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Noninvasive Transdermal Insulin Delivery Using Piston-Shaped PZT Transducers: In vivo Rabbits Evaluation

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Abstract

Noninvasive transdermal insulin delivery is investigated in this paper utilizing ultrasound transducers in order to improve the quality of life of Type 1 diabetic patients. This alternative technique is intended to replace the long-term dependence on multiple subcutaneous insulin injections. Different piston-shaped ultrasound transducers operating in the frequency range 100 – 1000 kHz were housed using silicone adhesive which included a reservoir to hold insulin during in vivo transdermal delivery. Twenty five local rabbits were divided into five groups and anesthetized using a combination of Ketamine hydrochloride and Xylazine to produce temporarily diabetic rabbit models during the period of experiments. Consisting of five rabbits in each experimental group, the control group (G0) did not receive ultrasound while exposure groups (G1-G4) received ultrasound for only ten minutes. Sweep driving mode of operation over a range of frequencies was applied to each exposure group with different frequency ranges. The swept frequency ranges were 100-200, 200-400, 400-650, and 650-1000 kHz for exposure groups G1, G2, G3, and G4, respectively. Initially, blood glucose level of rabbits (n = 25) was 157.2 ± 17.4 (mg/dl) and increased to 302.4 ± 78.1 mg/dl in one-hour period for the control group. In contrast, exposure groups (G1-G4) showed variable behaviors of glucose level reductions depending on driving frequencies with lowest value of 100.6 ± 17.9 (mg/dl) (G1) after one-hour from the starting of the ten minute exposure period. Compared to the control group, exposure groups showed reduction of blood glucose levels by 21.6%, 10.8%, 3.4%, and 3.7% for exposure groups G1, G2, G3, and G4, respectively, after twenty minutes from exposure period. The reduction of blood glucose levels continued till the end of the one-hour measurement period with maximum recorded reductions, compared to the control group, were 66.7%, 35.9%, 39.5% and 45% for groups G1, G2, G3, and G4, respectively. Ultrasound piston PZT transducers were found to facilitate insulin delivery across the skin of rabbits regardless of the driving frequency in the tested range from 100 to 1000 kHz. However, driving frequencies from 100 to 200 kHz were found to be the best facilitator of insulin delivery compared to other tested frequencies.

1. Introduction

Noninvasive transdermal delivery (NTD) of insulin may help improving the quality of life of type 1 diabetes patients. It is a preferable technique for diabetic patients over the traditional invasive and painful subcutaneous insulin injections [1]. Few researchers managed to utilize ultrasound transducers for transdermal insulin delivery [2-11]. Aside from sporadic studies which used different commercial, large, and heavy ultrasound equipments to deliver insulin across the skin [12], light-weight compact cymbal transducers were investigated using various ex vivo and in vivo animal experiments [13-20]. Blood glucose levels were decreased immediately after administration of insulin via ultrasound energy for 60 minutes of pulsed driving. Cymbal transducers were tested on different small and large animals with blood glucose levels decrease by 49%, 46%, and 60% from normal baseline levels for pigs, rabbits, and rats, respectively [13,14,16-18]. These results were achieved for different administration of ultrasound periods and blood glucose testing intervals, with the operating frequency of the cymbal transducers being fixed at 20 kHz [1, 21-23]. Since the biological mechanisms of insulin delivery across the skin is not yet known, the need to test more operating frequencies is required to uncover the optimal frequency that may facilitate and enhance the permeability of skin layers for best possible delivery of insulin molecules to the blood stream. The outer layer of the skin (stratum corneum) attributes mainly to the low permeability of the skin to transdermal drug delivery. Ultrasound energy, however, was found to facilitate the transportation of insulin across the condensed keratinocytes of the stratum corneum. Low frequency ultrasound was believed to only facilitate the drug delivery across skin layers due to microbubbles generation within these layers, which allows water channels to be produced within the lipid bilayers [24-28]. Possible mechanisms of transdermal insulin delivery utilizing ultrasound include cavitation, thermal effects, generation of convective velocities, and mechanical effects [29]. Experimental results propose that amid all the ultrasound-related facts, cavitation plays the

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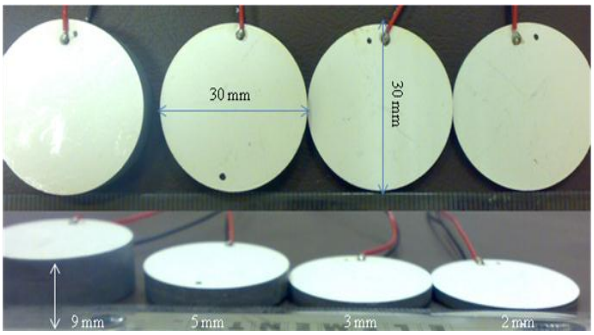
dominant role in ultrasound mediated drug delivery with low-frequency being more effective [30]. Based on the positive results from literature [13,14,16-18], the purpose of this study was to determine the feasibility of ultrasound mediated transdermal delivery of insulin in vivo specifically, using a portable ultrasound piston shaped device operating at frequencies from 100 to 1000 kHz.

2. Materials and Methods

2.1. Ultrasound transducers and driving setup:

Different piston-shaped ultrasound transducers, fabricated using Lead Zirconate Titanate 4 (PZT-4) material, were used through this study (Piezo Kinetics, Inc., Bellefonte, PA, USA). PZT-4 was chosen because of its high failure voltage threshold compared to ceramics with similar efficiency. Table 1 show physical properties and driving conditions of used PZT transducers. With fixed outer diameter of 30.0 mm, the thicknesses, which determine their resonance frequencies, vary from 9.0 to 2.0 mm. However, the under-water (i.e. loaded transducer) resonance frequency is lower than its tabulated in-air resonance frequency due to extra mass loading on the faces of transducers [31]. To prepare the piston-shaped transducers for animal experiments, a silicone adhesive material (Diya®, Amman, Jordan) was molded to build a proper housing for the transducers that holds insulin during in-vivo animal experiments. The silicone was molded into 33.0 mm cylindrical shape and left to cure overnight. The cured silicone mold was reshaped into a hollow cylinder to incase the transducer. Ultrasound transducers were then housed using these cured pieces of silicone in a way that created a reservoir for insulin loading during animal experiments. Figure 1 shows actual photos of the transducers before and after building the appropriate silicone housing with insulin reservoir for in vivo animal experiments. The driving conditions of these transducers were chosen to span the range from 100 kHz to 1 MHz. Continuous driving mode of operation was selected with variable sweeping frequencies. The driving signals' frequencies were continuously varied from a specific lower frequency to a higher frequency (sweep frequency range) during ten seconds duration (sweep duration). The continuous driving period of the transducers (insulin delivery period or exposure period) was set to 10 minutes for rabbit experiments. To drive the piston transducer, a radio frequency (RF) signal was generated by a sweep function generator (BK Precision model 4017A, Yorba Linda, CA, USA) and amplified by an RF amplifier (Model 25A250, Amplifier Research, Souderton, PA, USA) as shown in Figure 2. The sweep period, frequency range, and output RF signal from the sweep function generator were monitored using an oscilloscope (Tektronix TDS 1002 B, Beaverton, OR, USA). For the experiments, the function generator operated at sweep mode with frequencies listed in Table 1 with sweep duration of 10 seconds, while the amplifier electrical output power was set to 4 W. Swept ultrasound was used to avoid heat build-up that may harm the transducers and the animals skin.



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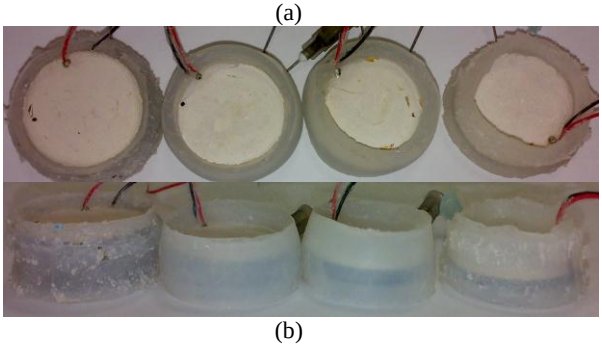


(b)

Figure 1: Upper and side views of the PZT transducers (a) before and (b) after building the housing.

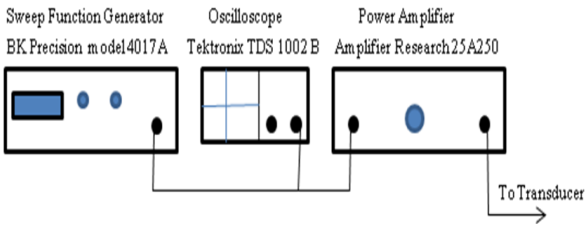


Figure 2: Schematic diagram of the driving setup of the ultrasound transdcuers. The sweep function generator feeds the sweep signal to an oscilloscope and to the power amplifier input port. The amplified signal is fed to the PZT transducer.

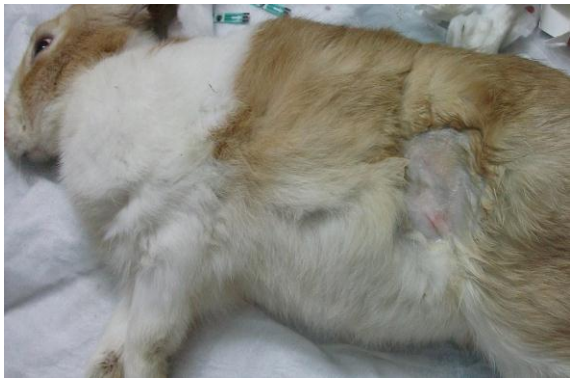
Table 1: Physical properties of PZT transducers and driving conditions for animal experiments.

Physical properties			Driving conditions		
Thickness (mm)	O.D. (mm)	Resonance freq (kHz)	Sweep freq range (kHz)	Sweep period (s)	Driving period (min)
9.0	30.0	225.8	100 – 200	10.0	10.0
5.0		406.4	200 – 400		
3.0		677.0	400 – 650		
2.0		1016.0	650 - 1000		

3. Animal experiments

The animals were anesthetized by procedures approved by the Animal Care Committee at the Hashemite University. Twenty five rabbits (1.5–2.5 kg) obtained from the local market were divided into five experimental groups. The first group (control group, G0) did not receive ultrasound exposure while the rest groups (G1-G4) were exposed to ultrasound using different sweep driving

frequencies as explained in table 2. Insulin was held between the transducer and the skin during the 10 minutes exposure period. Each animal was pre-anesthetized intramuscularly with a combination of Ketamine hydrochloride (40 mg/kg, TEKAM 50 mg/ml, HIKMA Co., Amman, Jordan) and Xylazine (10 mg/kg, XYLA-JECT 20 mg/ml, Adwia Co. S.A.E., 10th of Ramdan City, Egypt). The abdomen areas of the rabbits were shaved using an electric shaver, and a depilatory agent was applied to the skin of rabbits to eliminate any remaining hair. A square shaped medical plaster with squared hollow in the middle was bonded over the shaved area. A double face foam tape was fixed over the medical plaster to hold the transducer with its housing close to the rabbit's skin. The transducer was then easily attached to the foam tape. Two 16 G needles were inserted via the silicone housing to the reservoir between the face of the transducer and the shaved skin (Figure 3). The reservoir was filled with 4 ml of insulin (Mixtard® 30, Novo Nordisk, Denmark) using a 10 ml syringe attached to one of the needles. The other needle allowed trapped air to escape while filling the reservoir. At the beginning of the experiment, blood samples (0.3 ml) were collected from the ear vein of each rabbit for a baseline glucose level analysis. The glucose level (mg/dl) in the blood was determined using Gluco-Track® (Teco Diagnostics, CA, USA) blood glucose monitoring system. During each experiment, multiple blood samples (2–4 each time) were taken every 10 minutes for 60 minutes. Additionally, an examination of the rabbit's skin was performed after exposure to look for visible lesions on the skin surface. Visual inspection of the ultrasound exposed rabbit's skin did not indicate any visible damage or change to the skin.



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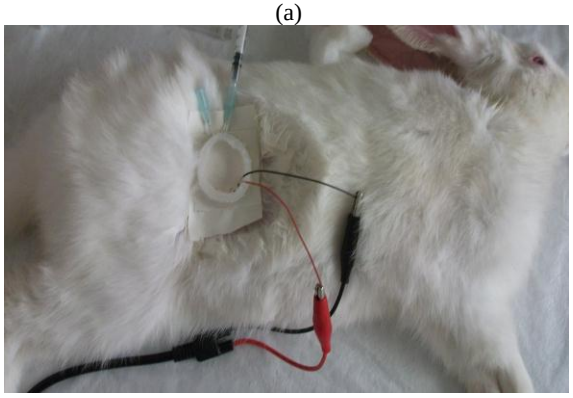

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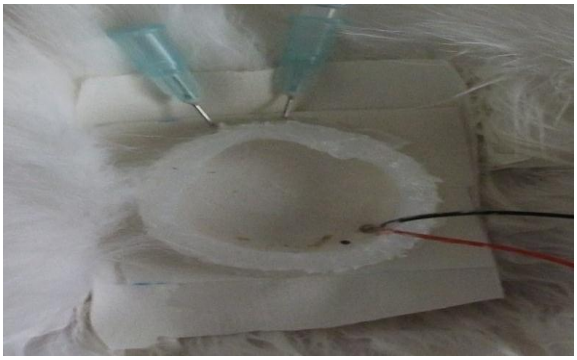

(c)

Figure 3: Photos of anesthetized local rabbits showing (a) the shaved abdominal area before fixing the transducer. A square-hollowed plaster is attached to the shaved area (b) that used to fix the transducer using double faced foam tape (c), where two needles are fixed to the housing for instant injection of insulin during experiments.

Table 2: Experimental groups of temporarly diabetic rabbits.

Experimental Group	Ultrasound frequncy range (kHz)	Transducer thikness (mm)	Number of Animals
G0	No ultrasound	--	5
G1	100 – 200	9.0	
G2	200 – 400	5.0	
G3	400 – 650	3.0	
G4	650 – 1000	2.0	

4. Results

Results of ultrasound mediated transdermal insulin delivery in temporarily diabetic rabbits for the five groups are graphed (Figure 4) as the change in the blood glucose level during the 60 minutes period experiments in terms of the mean and standard deviation error. After the rabbits were anesthetized, the average initial glucose level (zero time glucose level or base line) of the rabbits (n = 25) was 157.2 ± 17.4 (mg/dl). To normalize results to the base line value, the zero time glucose level value of each experimental group was subtracted from actual glucose level points to eliminate differences between groups' starting point (zero time point). Data was graphed in Figure 4 showing zero time point with zero glucose level for all groups. For the control group (G0), the glucose level increased to 173.4 mg/dl in 1 hour period. This increase of glucose level was measured immediately after the anesthesia of each rabbit. In contrast, exposure groups (G1-G4) showed lower increase of glucose levels after the 10 minutes delivery period with 45.0 mg/dl as the maximum increase (G3) at the 40 minutes time point (Figure 4). Exposure groups showed variable behaviors of glucose level reduction depending on driving frequencies with lowest value of -50.0 mg/dl (G1) after 1 hour from the starting of the ultrasound-mediated insulin delivery compared to the base line. To determine the statistical significance between the results in Figure 4 of the exposed groups (G1–G4) and control group (G0) at the 10 min increment time points, ANOVA was used to analyze this data (Table 3). The analysis showed that the results were statistically significant at a p-value of 0.05 or less for all time points except for the first point of group G2.

Table 3: p-values for the in-between control group (G0) and exposure groups (G1-4) over 60 minutes period.

Time (min)	Between groups p-values			
	G0 & G1	G0 & G2	G0 & G3	G0 & G4
10.0	0.0017	0.0944	0.0016	0.0007
20.0	0.0080	0.0146	0.0011	0.0009
30.0	0.0072	0.0112	0.0063	0.0016
40.0	0.0041	0.0253	0.0067	0.0006
50.0	0.0014	0.0178	0.0066	0.0002
60.0	0.0018	0.0066	0.0030	0.0002

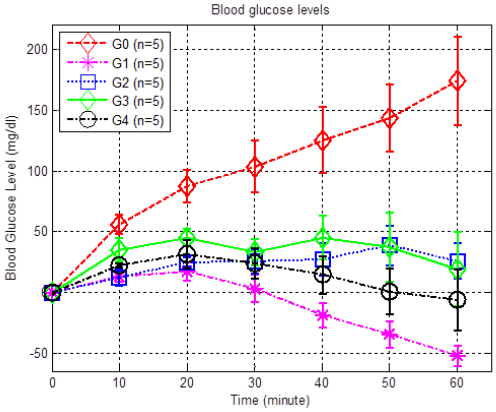

Figure 4: The change in blood Glucose concentrations for the temporarily diabetic rabbit model showing the control group (G0) and exposure groups (G1-G4).

5. Discussion

Both Figure 4 and Table 3 show actual blood glucose level (BGL) concentrations (mg/dl) over the one-hour recording period in ten-minute steps. Control group (G0) showed rapid glucose level increase from 129.0 ± 7.6 mg/dl at the zero time point to 302.4 ± 78.1 mg/dl at the sixty-minute point. Rapid linear increase was noticed during the first 20 minutes with almost linear increase after that but with different sloped line till the end of the one-hour experiment. With assumed linear increase starting from the twenty-minute time point, the slope was 2.12 mg/dl.min for the rest of timing points (from 20 to 60 minutes). Exposure groups, on the other hand, showed gradual increase to reach peak values at the twenty-minute time points with sharp linear reductions after that for both groups G1 and G4. Linear fitting showed that the slopes of these lines were -1.756 and -0.984 mg/dl.min for groups G1 and G4, respectively. However, groups G2 and G3 showed different behaviors. Under the same assumption of linear relationship (from 20 to 60 minutes period) for groups G2 and G3, calculated slopes of these assumed lines were 0.146 and -0.476 mg/dl.min, respectively. Compared to base line level, exposure group G1 showed an increase in blood glucose level during the ten-minute exposure period and continued to increase till the twenty-minute time point. BGL decreased linearly after that till the end of the experiments. Exposure group G2, on the other hand, produced elevated BGLs during the exposure period and continued to increase relatively sharply till the twenty-minute time point. Unlike exposure group G1's behavior, exposure group G2 continued to increase slowly after that to reach a maximum elevated point at the fifty-minute time point with a noticeable decrease after that to end with elevated BGL value compared to the base line level. Exposure group G3 showed elevated BGL for the first twenty minutes and then reduced at the thirty-minute time point with unexpected increase at the forty-minute time point then reduced back to an elevated level compared to the base line level. Exposure group G4, however, showed close behavior to group G1 with elevated BGL during the first 20 minutes after the start of the exposure period and gradually decreased to reach base line level at the fifty-minute time point and continued to decrease after that.

To further analyze these data, Table 4 shows the percentages of BGLs compared to the control group at each time point. Exposure groups (G1-4) showed elevated percentage BGLs compared to the control group immediately before the ultrasound-mediated insulin delivery process (the first 10 minutes). This elevated percentage BGL was due to the timing differences between exposure groups and control group due to the preparation of rabbits after anesthesia. In fact, readings of BGL in the control group started immediately after anesthesia of animals while readings of exposure groups started after shaving and adjustment of the ultrasound transducer on top of the double face foam tape as previously pointed out. The preparation time was kept during all experiments to less than 15 minutes from the injection of anesthesia. Immediately after the 10 minutes delivery period, both groups G1 and G2 showed reduction of BGL by 10.4% and 2.5%, respectively. However, groups G3 and G4 showed elevated BGL after the delivery period. At twenty minutes from the beginning of exposure, the results showed reduction of BGL by 21.6%, 10.8%, 3.4%, and 3.7% for exposure groups G1, G2, G3, and G4, respectively. Using linear curve fitting of these data starting from the twenty-minute time point, the slopes of the lines were -1.139, -0.577, -0.929, and -0.995 mg/dl.min for groups G1, G2, G3, and G4, respectively. The reduction of BGL continued for the measuring period with maximum recorded reductions of 66.7%, 35.9%, 39.5% and 45% for groups G1, G2, G3, and G4, respectively. Figure 5 shows the behavior of BGL for each group compared to control group during the 1-hour recording period. Group G1 showed faster delivery of insulin followed by group G4, while both groups G2 and G3 showed almost the same behavior of delivery. These data proved that ultrasound facilitated insulin delivery across the skin of rabbits regardless of the driving frequency in the tested range from 100 to 1000 kHz. However, driving frequencies from 100 to 200 kHz were the best facilitator of insulin delivery compared to tested frequencies from 200 to 1000 kHz. Sweep frequencies from 650 to 1000 kHz were found comparable to group G1 behavior but with less amount of insulin delivery; while groups G2 and G3 were the least effective in delivery in this study.

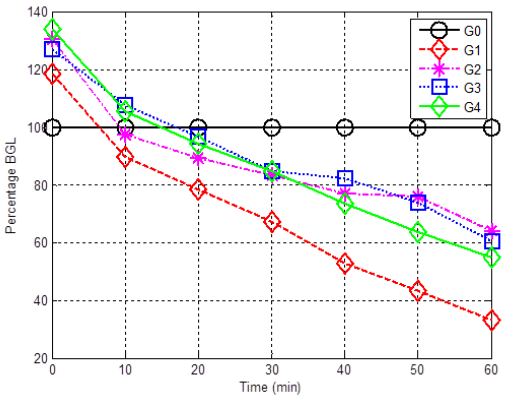

Figure 5: Percentages of exposure groups' BGL compared to control group (G0) over the course of recording period.

Table 4: Percentages of blood glucose concentrations of exposure groups compared to control group.

Time (min)	Percentages of blood glucose levels compared to control group			
	G1	G2	G3	G4
0.0	118.3%	130.2%	127.0%	133.8%
10.0	89.6%	97.5%	107.7%	105.4%
20.0	78.4%	89.2%	96.6%	94.3%
30.0	67.2%	83.5%	84.7%	84.7%
40.0	52.7%	76.9%	82.2%	73.5%
50.0	43.5%	76.0%	74.0%	63.8%
60.0	33.3%	64.1%	60.5%	55.0%

6. Conclusions

Piston transducers were found feasible in delivering insulin across the skin in a noninvasive manner. Compared to other transducers, piston transducers are cheaper, portable, smaller in size, and can be fabricated with predefined thicknesses for explicit frequency driving conditions. Specifically to this study, the sweeping mode of driving was used to reduce the driving periods on the resonance frequency which may lead to transducer failure under continuous driving conditions. Another reason for this choice is the excitation of a spectrum of frequencies that may lead to uncover the best driving frequency, or range of frequencies, for maximum delivery of insulin. Although, low frequency range (20 to 100 kHz) was suggested for drug delivery [12], piston transducers with driving frequencies from 100 kHz to 1.0 MHz gave good results with faster delivery when using driving frequencies from 100 to 200 kHz with the 9.0 mm thickness piston transducer.

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