Journal of Diabetes Science and Technology
Volume 3, Issue 3, May 2009

ORIGINAL ARTICLES

Noninvasive Ultrasonic Glucose Sensing with Large Pigs (~200 Pounds) Using a Lightweight Cymbal Transducer Array and Biosensors

Eun-Joo Park, M.S.,[1] Jacob Werner, V.M.D.,[2] Joshua Beebe,[3] Samantha Chan,[1] and Nadine Barrie Smith, Ph.D.[1,4]

Abstract

Background:

To prevent complications in diabetes, the proper management of blood glucose levels is essential. Since conventional glucose meters require pricking fingers or other areas of the skin, a noninvasive method for monitoring blood glucose levels is desired. Using a lightweight cymbal transducer array, this study was conducted to noninvasively determine the glucose levels of pigs having a similar size to humans.

Method:

In vivo experiments using eight pigs (~200 pounds) were performed in five groups. A cymbal array with four biosensors was attached to the axillary area of the pig. The array was operated at 20 kHz at special peak–temporal peak intensity (I_{sptp}) equal to 50 or 100 mW/cm^2 for 5, 10, or 20 minutes. After the ultrasound exposure, glucose concentrations of the interstitial fluid were determined using biosensors. For comparison, glucose levels of blood samples collected from the ear vein were measured by a commercial glucose meter.

Result:

In comparison, glucose levels determined by a cymbal array and biosensor system were close to those measured by a glucose meter. After a 20-minute ultrasound exposure at I_{sptp} = 100 mW/cm^2, the average glucose level determined by the ultrasound system was 175 ± 7 mg/dl, which is close to 166 ± 5 mg/dl measured by the glucose meter.

Conclusion:

Results indicate the feasibility of using a cymbal array for noninvasive glucose sensing on pigs having a similar size to humans. Further studies on the ultrasound conditions, such as frequency, intensity, and exposure time, will be continued for effective glucose sensing.

J Diabetes Sci Technol 2009;3(3):517-523

Author Affiliations: [1]Department of Bioengineering, The Pennsylvania State University, University Park, Pennsylvania; [2]Animal Resource Program, The Pennsylvania State University, University Park, Pennsylvania; [3]Department of Mechanical Engineering, The Pennsylvania State University, University Park, Pennsylvania; and [4]Graduate Program in Acoustics, The Pennsylvania State University, University Park, Pennsylvania

Abbreviations: (ANOVA) analysis of variance, (GOx) glucose oxidase, (I_{sptp}) special peak–temporal peak intensity, (PBS) phosphate-buffered saline, (PZT) lead zirconate–titanate, (RF) radio frequency

Keywords: cymbal transducer, glucose sensing, noninvasive, transdermal, ultrasound

Corresponding Author: Eun-Joo Park, M.S., Department of Bioengineering, The Pennsylvania State University, 205 Hallowell Building, University Park, PA 16802; email address *eup114@psu.edu*

Introduction

In the management of diabetes mellitus, the tight control of blood glucose concentration can reduce the risk of short- and long-term complications, including chronic renal failure, retinal damage, nerve damage, cardiovascular disease, and microvascular damage.[1,2] While a conventional method of blood glucose monitoring by pricking fingers or other skin locations is widely used, it has disadvantages as an invasive and painful technique, which often leads to noncompliance. Therefore, researchers have focused on developing alternatives capable of measuring glucose levels noninvasively or minimally invasively.[3–8]

Measuring glucose concentration of the interstitial fluid has shown to be an alternative to blood glucose measurements.[4,7,9-11] One of the devices used for measuring the glucose concentration of the interstitial fluid is a subcutaneous sensor such as the MiniMed continuous glucose monitoring system (Medtronic, Inc., Minneapolis, MN).[9] Depending on the model, the subcutaneous sensor can measure the glucose level every 1 to 5 minutes. However, it is still an invasive technique requiring a subcutaneous insertion of sensors. Also, it has some drawbacks, such as the need to replace sensors every 3 to 7 days and the interaction of biological components causing physiological clots.[12–14] Devices using microdialysis also measure the glucose concentration of the interstitial fluid.[4] Despite the advantage of providing a sufficiently precise measurement, devices of this technique are either available for physicians only or are too bulky to wear during daily activities. In addition to these techniques, transdermal extraction of glucose in the interstitial fluid using ultrasound has been studied as a potential method of glucose sensing.[7,10,11] Based on the facts that ultrasound can permeate skin and that the glucose level of the interstitial fluid is highly correlated to the blood glucose level, ultrasound has been used in transdermal drug delivery and monitoring glucose concentrations.[7,10,11,15–20] After the ultrasound enhances skin permeability by cavitation, glucose in the interstitial fluid is diffused out due to the difference of concentration between the interstitial fluid and the phosphate-buffered saline (PBS) solution in a reservoir. Because this method utilizes glucose that is diffused naturally through the permeabilized skin, ultrasonic glucose sensing is considered a non-invasive modality.

To evaluate the practical use of an ultrasound system in diabetes management, a cymbal transducer array has been used in transdermal insulin delivery[15–17] and glucose sensing.[7] The cymbal transducer is a flextensional transducer with a compact structure.[18–20] For perspective human applications, a recent *in vivo* study has demonstrated the feasibility of using the cymbal array in ultrasonic transdermal insulin delivery on large animals having a similar size and weight to humans.[17] As a milestone to a preclinical application, this research has investigated the feasibility of using the cymbal array in ultrasonic glucose sensing by conducting *in vivo* experiments with large pigs having a similar size and weight to humans (~200 pounds). Furthermore, the reliability of transdermal glucose sensing has been explored in different ultrasound exposure conditions.

Materials and Methods

Cymbal Transducer Array

The cymbal transducer is a class V flextensional transducer with an adjustable resonance frequency between 1 and 100 kHz.[21–23] In the design of a cymbal transducer, the lead zirconate–titanate (PZT) ceramic disk is sandwiched between metal end caps having a shallow cavity beneath the surface (**Figure 1A**). The radial motion of the ceramic disk is converted into the axial motion of end caps, which is the fundamental vibration mode of the transducer. To construct the cymbal transducer, the piezoelectric disk, having a diameter of 12.7 mm and a thickness of 1 mm, was made from PZT-4 (Piezokinetics, Inc., Bellefonte, PA). The end caps, made of 0.25-mm-thick brass, were bonded on the ceramic disk using Eccobond® (Emerson & Cuming, Billerica, MA) epoxy. In order to have the biosensors located between the array and the skin without disturbing the ultrasound field, four cymbal transducers were connected in a two-by-two pattern to construct an array arrangement and encased in polyurethane (CPD 9150, Epoxical, St. Paul, MN). The two-by-two array used in this study has the size of $38 \times 38 \times 5$ mm^3 (**Figure 1B**).

In order to drive the array, a radio frequency (RF) signal was generated by a function/arbitrary waveform generator (Model 33250A, Agilent, Santa Clara, CA) and amplified by an RF amplifier (Model 25A250, Amplifier Research, Souderton, PA). Using an external tuning network, the electrical impedance of the array was tuned to the output impedance of the amplifier. Using a digital oscilloscope (Model 54622A, Agilent), the pulse duration

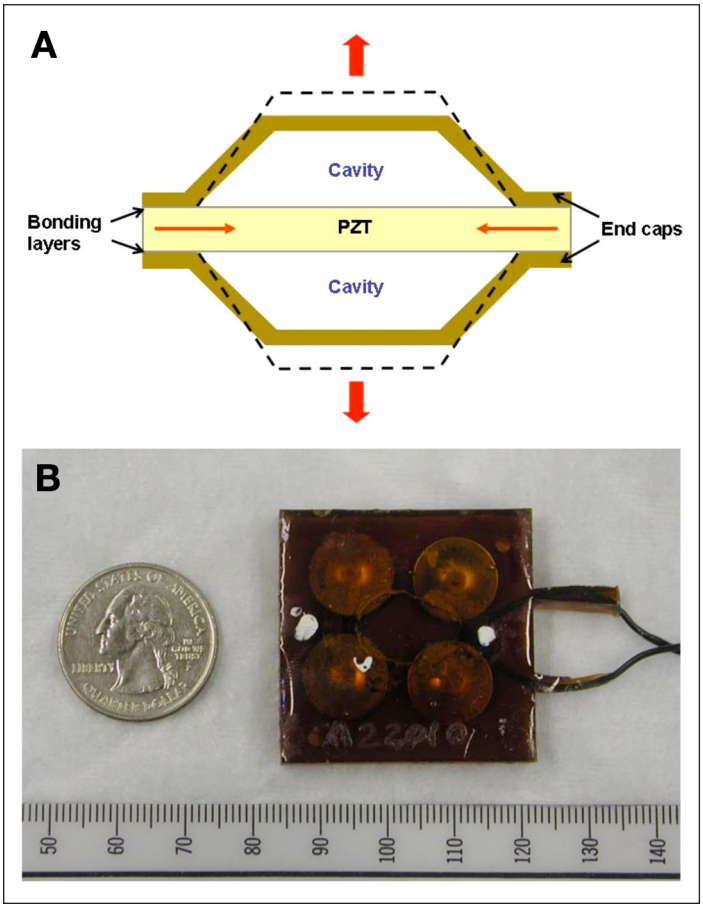

Figure 1. (A) A cymbal transducer consisting of a ceramic disk sandwiched between two brass end caps resonates at 20 kHz. Displacement of the ceramic disk is converted into the axial displacement of end caps. Dashed lines represent the flexing of end caps, and arrows indicate the motion. **(B)** A lightweight cymbal array was constructed using four cymbal transducers, which were connected in a 2 × 2 pattern and encased in a polymer.

and repetition period of the RF signal were monitored. In this research, the array was operated at a frequency of 20 kHz with a pulse duration of 200 ms and a pulse repetition period of 1 second based on a previous study in rats.[7]

According to the exposimetry guidelines established by the American Institute of Ultrasound in Medicine, the acoustic pressure field of the array was determined with a calibrated miniature omnidirectional reference hydrophone (Model TC4013, S/N: 5199093, Reson, Inc., Goleta, CA).[24,25] The cymbal array was submerged in a water tank (51 × 54 × 122 cm^3), which was made almost anechoic by placing 1.27-cm-thick rubber sound-absorbing material around its wall. To avoid cavitation effects, the tank was filled with distilled water degassed by a custom-made degasser to reduce the dissolved oxygen content to 1–2 ppm. The position of the hydrophone was controlled precisely by a Velmex positioning system (Velmex Inc., East Bloomfield, NY). In order to obtain the acoustic pressure profile, automatic scannings over the 50 × 50-mm^2 area with a 1-mm step size were performed by the computer-controlled exposimetry positioning system. Based on three scannings over a plane 2 mm away from the array surface, spatial peak–temporal peak (I_{sptp}) intensity was determined in a mean and standard deviation. The intensities of the cymbal transducer array were 101.2 ± 2.3 and 50.9 ± 1.8 mW/cm^2.

Biochemical Glucose Sensor

Based on the property of electrochemical biosensors that produce electrical signals proportional to the change of physiological parameters, enzyme-based biosensors were used as glucose sensors. In the presence of glucose oxidase enzyme (GOx), glucose reacts with oxygen (O_2) and water (H_2O) to produce gluconic acid and hydrogen peroxide (H_2O_2)[26]:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOx}} \text{Gluconic Acid} + H_2O_2.$$

When a positive electric potential is applied under the presence of platinum as a catalyst, two hydrogen peroxide molecules are oxidized into oxygen, water, and four electrons:

$$2H_2O_2 \xrightarrow{\text{Pt}} 2O_2 + 2H_2O\ 4e^-.$$

The current produced in this reaction is proportional to the concentration of hydrogen peroxide, which is proportional to the glucose concentration.

In this study, the biosensors were fabricated based on polymer-thick film electrodes acquired from Conductive Technologies Inc. (York, PA). The biosensor consists of three electrodes: working, counter, and reference (**Figure 2**). To deposit platinum on the working electrode, platinic acid of 25 m*M* (Sigma-Aldrich Corp., St. Louis, MO) in 0.1 *M* NaCl solution was placed over three electrodes. Using a potentiostat (Model 283, Princeton Applied Research, Oak Ridge, TN), a voltage of –0.5 volt between working and counter electrodes was applied for 60 minutes. Glucose oxidase (1,000,000 unit/gram; Sigma-Aldrich Corp.) was immobilized by polyethylene glycol hydrogel. After polyethylene glycol diacrylate (a molecular weight of 575; Aldrich Corp.) was diluted with distilled water to produce a 20% solution, a liquid photoinitiator (2-hydroxy-2-methyl-1-phenyl-1-propanone; Ciba Specialty Chemicals, Tarrytown, NY) and glucose oxidase were mixed with the solution. Three electrodes were covered with the solution and exposed to 6-watt,

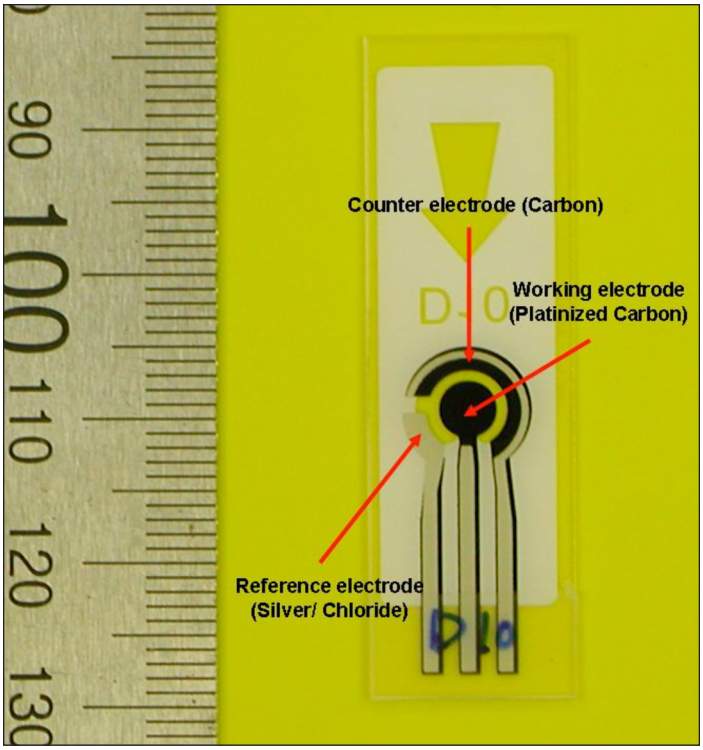

Figure 2. An enzyme-based electrochemical biosensor. The sensor consists of three electrodes: working/sensing, counter, and reference.

365-nm ultraviolet light for 20 seconds in air. To determine the unknown glucose concentration of the interstitial fluid, each biosensor was calibrated using a concentration curve created by a series of glucose solutions—0, 50, 100, 150, 200, and 300 mg/dl. The glucose solutions were prepared by dissolving glucose (dextrose anhydrous; VWR, West Chester, PA) in 0.9% PBS solution.

In Vivo Experiments in Pigs (~200 Pounds)

For perspective future human application, *in vivo* studies in large pigs were designed to determine the feasibility of the lightweight cymbal array for noninvasive ultrasonic glucose monitoring. Eight Yorkshire pigs (~200 pounds) obtained from The Pennsylvania State University Swine Center were used in five groups according to the ultrasound exposure conditions. All animal experiments were conducted with procedures approved by the Institutional Animal Care and Use Committee at Pennsylvania State University (IACUC No. 21998).

After preanesthesia 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), a pig was fitted with an intravenous 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 a surgical depth via inhalant isoflurane (Isothesia, Abbott Laboratories, Abbott Park, IL). Using an electric shaver and a depilatory agent, hair on the axillary area of pigs in lateral recumbency was removed. Experiments were performed on the same location of each pig. After hair removal, the cymbal array, with double layers of a watertight standoff made of 1-mm-thick plastic, was attached to the skin using tissue glue (Vetbond®, 3M, St. Paul, MN). **Figures 3A** and **3B** show the layout of the glucose sensor incorporated with the cymbal array and a photograph of an ultrasonic glucose-sensing experiment, respectively. Tissue glue was applied on the standoff, which was attached to the skin. Four biosensors were placed between two layers of the standoff. The reservoir within the standoff was filled with a 0.9% PBS solution through a small hole in the back of the array. Since it was assumed that the concentration of glucose diffused out to the reservoir depends on the volume of the PBS solution, the volume was kept at 2.25 ml for all experiments. In order to prevent the disruption of ultrasound transmission, bubbles from the solution in the reservoir were eliminated.

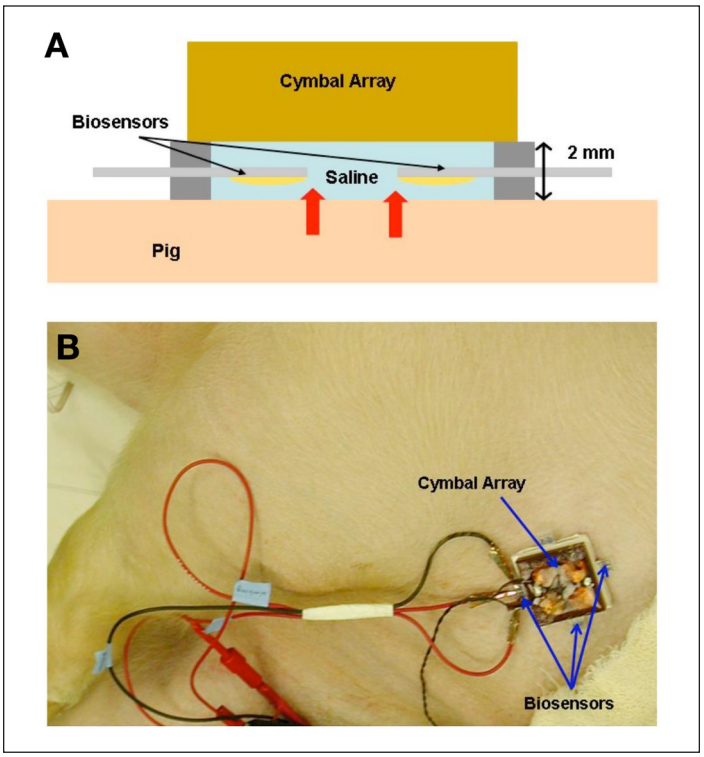

Figure 3. (A) Layout of the glucose sensor incorporated with the cymbal array placed on the skin surface. After fixing the hydrogel of glucose oxidase on the biosensor, four biosensors were placed between the cymbal array and the standoff. **(B)** An ultrasonic glucose-sensing experiment with a pig placed in a lateral recumbent position. The reservoir within the standoff was filled with PBS through a small hole in the back of the array.

To permeabilize the skin for the extraction of glucose in the interstitial fluid, the cymbal array was operated at a frequency of 20 kHz with an intensity of I_{sptp} = 100 or 50 mW/cm^2 for 5, 10, or 20 minutes (**Table 1**). After connecting the biosensors to the potentiostat at the end of the ultrasound exposure, the current from the electrochemical reaction on the biosensors was measured by applying a voltage of 0.7 volt to the working electrode against the counter electrode. Using the potentiostat (Model 283, Princeton Applied Research), voltage applied to the working electrode was increased from 0 to 0.7 volt with an increasing ratio of 10 mV/sec. Using the potentiostat control program (Electrochemistry PowerSuite™ v. 2.1.1, Princeton Applied Research), the current was recorded on a computer. Based on biosensor calibration results and the linear relationship between the current and the glucose concentration, glucose levels in the interstitial fluid were determined. For the comparison to glucose levels determined using the cymbal array and biosensor system, blood samples were collected at the same time when currents from the biosensors were measured. The glucose level of the blood sample was measured by an Accu-Chek™ blood glucose meter (Roche Diagnostics Co., Indianapolis, IN) and an i-STAT portable clinical analyzer (Model 200, Abbott Laboratories). Results were presented as a mean and standard error. An analysis of variance (ANOVA) was used to analyze the statistical significance of differences among the means of groups. A *p* value was used to determine if between-group differences were significantly greater than chance. For all data, a single or double asterisk was used if the *p* value was higher than the 0.01 or 0.05 level of significance, respectively.

Table 1.
Detailed Information of Ultrasound Exposure Conditions[a]

Acoustic intensity, I_{sptp}	Exposure time (minutes)
100 mW/cm^2	20
	10
	5
50 mW/cm^2	10
	5

[a] The cymbal array was operated at a frequency of 20 kHz with a pulse duration of 200 ms and a pulse repetition period of 1 second.

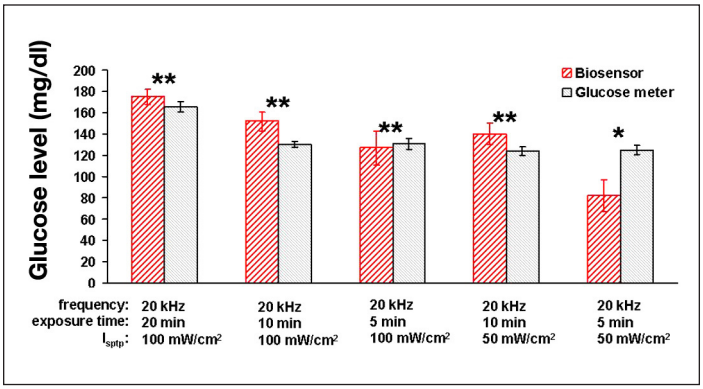

Figure 4. Glucose concentrations of interstitial fluid were determined using an ultrasound system consisting of the cymbal array and enzyme-based biosensors. In comparison to the measurement by an Accu-Check glucose meter and an i-STAT analyzer, the result of ultrasonic measurement with I_{sptp} = 100 mW/cm^2 for 10 minutes has shown reliable accuracy.

Results

Based on the concentration curve created previously for each biosensor, glucose concentrations of the interstitial fluid were determined using the current measured by the biosensors. For each exposure condition, results of ultrasonic glucose sensing using the cymbal array and biosensor system were graphed and compared to results of a conventional glucose meter (**Figure 4**).

With an acoustic intensity of I_{sptp} = 100 mW/cm^2, the glucose level determined by the biosensors after 20 minutes of ultrasound exposure was 175 ± 7 mg/dl, whereas the level measured by the conventional glucose meter was 166 ± 5 mg/dl. After 10 minutes of ultrasound exposure, glucose levels determined by the biosensor and glucose meter were 152 ± 9 and 130 ± 3 mg/dl, respectively. In comparison to the glucose level (131 ± 5 mg/dl) measured by a conventional glucose meter, the glucose concentration of the interstitial fluid was 127 ± 16 mg/dl, which was determined by the biosensors after 5 minutes of ultrasound exposure. Glucose levels of the interstitial fluid after ultrasound exposure with an intensity of I_{sptp} = 50 mW/cm^2 for 10 and 5 minutes were determined by the biosensors as 140 ± 10 and 82 ± 15 mg/dl, respectively. Compared to results of the biosensors, glucose levels measured by the conventional glucose meter after 10 and 5 minutes of exposure were 124 ± 4 and 125 ± 4 mg/dl.

In **Figure 4**, asterisks above the data bar were used if there was no statistical difference between the ultrasonic glucose-sensing method and the conventional method based on an ANOVA with a 0.05 or 0.01 level of significance. For the intensity of I_{sptp} = 100 mW/cm^2, glucose concentrations determined by the ultrasound system were statistically the same as the value determined by

the glucose meter. In addition, results of the 10-minute ultrasound exposure at the intensity of I_{sptp} = 50 mW/cm^2 showed no difference between two measurements. At the end of each experiment, the skin area exposed to the ultrasound was examined for a skin reaction and no skin irritation was found from all experiments.

Discussion

In several studies, researchers have demonstrated that ultrasound can permeabilize skin to enhance the transdermal delivery of drugs.[17–20] Results of cavitation have been suggested as the main mechanism of ultrasound-mediated transdermal drug delivery, although it has not been completely understood.[27–30] In addition to drug delivery, transdermal extraction of glucose in the interstitial fluid by ultrasound has shown potential in becoming an alternative technique of glucose measurement.[7,10,11]

This research was designed based on the effects of ultrasound that enhances skin permeability and the mechanism of glucose diffusion due to concentration differences. Previously, *in vivo* experiments of transdermal glucose sensing on rats were conducted using a lightweight cymbal transducer array and electrochemical biosensors, and results have demonstrated the feasibility of using the cymbal array in ultrasonic glucose measurement. Additionally, results have shown a reliable accuracy of this technique compared to the conventional method.[7]

In perspective to human application, the purpose of this research was to demonstrate the feasibility of the cymbal array in ultrasonic glucose monitoring on large pigs having a similar size and weight to humans. As the initial study of an *in vivo* pig experiment, results of ultrasound exposure at the same exposure condition (I_{sptp} = 100 mW/cm^2, 20 minutes) used in a previous study[7] have shown that an ultrasound system consisting of a cymbal array and biosensors was able to noninvasively determine the glucose concentration on large animals having a similar size to humans. To explore the reliability of the ultrasound system for glucose sensing, further experiments have been performed with different ultrasound exposure conditions. From statistical analyses, results of ultrasound exposure with I_{sptp} = 100 mW/cm^2 for 10 and 5 minutes have shown a reliable accuracy compared with results of conventional glucose meter measurements.

Results presented herein have demonstrated the feasibility of using a cymbal transducer array for ultrasonic glucose sensing on large animals for preclinical applications. Furthermore, results of ultrasonic glucose measurements with 100 mW/cm^2 (I_{sptp}) have indicated that the ultrasound system using the cymbal array has been able to determine the glucose concentration in short exposure time. To achieve the development of safe, painless, and continuous monitoring of glucose concentration, the accuracy of ultrasonic glucose measurement will be investigated for other operating conditions of the cymbal array, such as frequency, pulse duration, and pulse repetition. In addition, the dynamics of correlation between glucose in the blood and interstitial fluid will be investigated.

Funding:

This work was supported by the Department of Defense Technologies for Metabolic Monitoring Award Number W81XWH-05-1-0617.

References:

1. The Diabetes Control and Complications Trial Research Group (DCCT). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes-mellitus. N Engl J Med. 1993;329(14):977-86.
2. Wickramasinghe Y, Yang Y, Spencer SA. Current problems and potential techniques in *in vivo* glucose monitoring. J Fluoresc. 2004;14(5):513-20.
3. Sekkat N, Naik A, Kalia YN, Glikfeld P, Guy RH. Reverse iontophoretic monitoring in premature neonates: feasibility and potential. J Controlled Release. 2002;81(1-2):83-9.
4. Heinemann L. Continuous glucose monitoring by means of the microdialysis technique: underlying fundamental aspects. Diabetes Technol Ther. 2003;5(4):545-61.
5. Caduff A, Hirt E, Feldman Y, Ali Z, Heinemann L. First human experiments with a novel non-invasive, non-optical continuous glucose monitoring system. Biosens Bioelectron. 2003;19(3):209-17.
6. Ballerstadt R, Polak A, Beuhler A, Frye J. In vitro long-term performance study of a near-infrared fluorescence affinity sensor for glucose monitoring. Biosens Bioelectron. 2004;19(8):905-14.
7. Lee S, Nayak V, Dodds J, Pishko M, Smith NB. Glucose measurements with sensors and ultrasound. Ultrasound Med Biol. 2005;31(7):971-7.
8. Iguchi S, Kudo H, Saito T, Ogawa M, Saito H, Otsuka K, Funakubo A, Mitsubayashi K. A flexible and wearable biosensor for tear glucose measurement. Biomed Microdevices. 2007;9(4):603-9.
9. Mastrototaro J. The MiniMed Continuous Glucose Monitoring System (CGMS). J Pediatr Endocrinol Metab. 1999; 12 Suppl 3:751-8.
10. Mitragotri S, Coleman M, Kost J, Langer R. Analysis of ultrasonically extracted interstitial fluid as a predictor of blood glucose levels. J Appl Physiol. 2000;89(3):961-6.
11. Cantrell JT, McArthur MJ, Pishko MV. Transdermal extraction of interstitial fluid by low-frequency ultrasound quantified with 3H2O as a tracer molecule. J Pharm Sci. 2000;89(9):1170-9.

12. Garg S, Zisser H, Schwartz S, Bailey T, Kaplan R, Ellis S, Jovanovic L. Improvement in glycemic excursions with a transcutaneous, real-time continuous glucose sensor. Diabetes Care. 2006;29(1):44-50.

13. Wong LJ, Buckingham BA, Kunselman B, Istoc E, Leach J, Purvis R. Extended use of a new continuous glucose monitoring system with wireless data transmission in children with type 1 diabetes mellitus. Diabetes Technol Ther. 2006;8(2):139-45.

14. Weinstein RL, Bugler JR, Schwartz SL, Peyser TA, Brazg RL, McGarraugh GV. Accuracy of the 5-day Freestyle Navigator Continuous Glucose Monitoring System: comparison with frequent laboratory reference measurements. Diabetes Care. 2007;30(5):1125-30.

15. Lee S, Newnham RE, Smith NB. Short ultrasound exposure times for noninvasive insulin delivery in rats using the lightweight cymbal array. IEEE Trans Ultrason Ferroelectr Freq Control. 2004;51(2):176-80.

16. Lee S, Snyder B, Newnham RE, Smith NB. Noninvasive ultrasonic transdermal insulin delivery in rabbits using the light-weight cymbal array. Diabetes Technol Ther. 2004;6(6):808-15.

17. Park EJ, Werner J, Smith NB. Ultrasound mediated transdermal insulin delivery in pigs using a lightweight transducer. Pharm Res. 2007;24(7):1396-401.

18. Tachibana K, Tachibana S. The use of ultrasound for drug delivery. Echocardiography. 2001;18(4):323-8.

19. Pitt WG, Husseini GA, Staples BJ. Ultrasonic drug delivery--a general review. Expert Opin Drug Deliv. 2004;1(1):37-56.

20. Mitragotri S. Healing sound: the use of ultrasound in drug delivery and other therapeutic applications. Nat Rev Drug Discov. 2005;4(3):255-60.

21. Newnham RE, Xu QC, Yoshikawa S. Transformed stress direction acoustic transducer. J Acoust Soc Am. 1991;91:1198-9.

22. Newnham RE, Xu QC, Yoshikawa S. Metal-electroactive ceramic composite actuators. J Acoust Soc Am. 1994;97:3215.

23. Maione E, Shung KK, Meyer RJ Jr, Hughes JW, Newnham RE, Smith NB. Transducer design for a portable ultrasound enhanced transdermal drug delivery system. IEEE Trans Ultras Ferroelectr Freq Control. 2002;49(10):1430-6.

24. AIUM: Bioeffects and safety of diagnostic ultrasound. Rockville, MD: American Institute of Ultrasound in Medicine; 1993.

25. IEEE Standards Board. New York: IEEE Standard on Piezoelectricity; 1988.

26. Wilkins E, Atanasov P. Glucose monitoring: state of the art and future possibilities. Med Eng Phys. 1996;18(4):273-88.

27. Mitragotri S, Edwards DA, Blankschtein D, Langer R. A mechanistic study of ultrasonically-enhanced transdermal drug delivery. J Pharm Sci. 1995;84(6):697-706.

28. 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(10):1190-92.

29. Guzman HR, McNamara AJ, Nguyen DX, Prausnitz MR. Bioeffects caused by changes in acoustic cavitation bubble density and cell concentration: a unified explanation based on cell-to-bubble ratio and blast radius. Ultrasound Med Biol. 2003; 29(8):1211-22.

30. Schlicher RK, Radhakrishna H, Tolentino TP, Apkarian RP, Zarnitsyn V, Prausnitz MR. Mechanism of intracellular delivery by acoustic cavitation. Ultrasound Med Biol. 2006;32(6):915-24.