

● *Original Contribution*

## GLUCOSE MEASUREMENTS WITH SENSORS AND ULTRASOUND

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**Abstract**—Accurate monitoring of the blood glucose level in diabetics is essential in preventing complications. Generally, conventional over-the-counter glucose meters require frequent painful finger punctures to obtain samples, which makes a noninvasive method preferable. The purpose of this study was to demonstrate that glucose levels can be measured transdermally with the combination of the low-profile cymbal array and an electrochemical glucose sensor consisting of amperometric electrodes and a novel glucose oxidase hydrogel. Interstitial fluid glucose concentrations can be determined with the electrochemical glucose sensor after the skin is made permeable to glucose by ultrasound (US) (20 kHz) with the thin (< 7 mm) and light (< 22 g) cymbal array. Using this array to deliver insulin into hyperglycemic rats, our previous experiments demonstrated that blood glucose levels would decrease 233.3 mg/dl with 5 min of US exposure. With the sensor and array, our goal was to determine the glucose levels of hyperglycemic rats noninvasively and evaluate the possible bioeffects. A total of 12 anesthetized rats were placed into two groups (US exposure group and control group) and the array ( $I_{SPTP} = 100 \text{ mW/cm}^2$ ) with a saline reservoir operating for 20 min was affixed to the abdomen. The array was removed and an electrochemical glucose sensor was placed on the exposed area to determine the glucose concentrations through the skin. Comparison was made using a commercial glucose meter with the blood collected from a jugular vein. The average blood glucose level determined by the sensor was  $356.0 \pm 116.6 \text{ mg/dl}$ , and the glucose level measured by the commercial glucose meter was  $424.8 \pm 59.1 \text{ mg/dl}$ . These results supported the use of this novel system consisting of the electrochemical glucose sensor and the cymbal array for glucose monitoring. (E-mail: nbs@enr.psu.edu)  
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**Key Words:** Ultrasound, Noninvasive, Glucose, Glucose monitoring, Electrochemical biosensor, Glucose oxidase hydrogel, Transdermal, Ultrasound safety.

### INTRODUCTION

Without proper blood glucose monitoring, diabetics can be unaware of their hyperglycemic state, which can lead to chronic complications such as heart disease, blindness, renal failure, peripheral vascular disease and limb amputation (American Diabetes Association 2001; Brink 2002; Gale 2001). Currently, over-the-counter glucose meters are used, which are widely available for determining blood glucose levels, but which require skin punctures to obtain blood samples several times a day. The dermis on the finger tip is the preferred site for obtaining a blood sample, because of its high capillary

density. However, the dermis is well-supplied with high-density sensory neurons, which makes the procedure painful. Numerous methods have been explored to find noninvasive and painless approaches to extract dermal interstitial fluid, which has a similar glucose concentration to that of plasma (Bantle and Thomas 1997).

Reverse iontophoresis and sonophoresis have been previously studied to extract interstitial fluid noninvasively (Potts et al. 2002; Tierney et al. 2001). Using a low-level electrical current, reverse iontophoresis extracts glucose in the fluid through the skin of the diabetics. Early research reported that the reverse iontophoresis device showed a 96% accuracy compared with a standard blood glucose meter over a 12-h period (Potts et al. 2002). With ultrasound (US) at 20 kHz, *in vivo* rat skin was permeabilized after 2 min of  $10 \text{ W/cm}^2$  (spatial-peak temporal-peak intensity,  $I_{SPTP}$ ) exposure. The error of the

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average glucose level determined by radiolabeled glucose was 42 mg/dl when the venous glucose level was higher than 200 mg/dl (Kost et al., 2000). Ultrasound (12-W reported intensity over a 0.8 cm<sup>2</sup> area of skin) has also been used with diabetics and the determined glucose levels were 95% accurate (Chuang et al. 2004). After the skin was made permeable to glucose by iontophoresis or US in these studies, an electrochemical biosensor that produces an electric signal proportional to the concentration of glucose was used to determine the glucose level in the interstitial fluid (Peura 1992; Russell et al. 2001; Sirkar and Pishko 1998; Tierney et al. 2001).

The ability of the cymbal array to make the skin permeable has been previously demonstrated by the enhanced transportation of insulin across *in vitro* human skin and *in vivo* rats and rabbits (Lee et al. 2004; Smith et al. 2003a, 2003b). Using Humulin<sup>®</sup>-R insulin and after 5 min of US exposure, elevated blood glucose levels in rats were significantly reduced from approximately 420 mg/dl to 160 mg/dl. This result demonstrated that the rat skin permeability to insulin was enhanced by utilizing the cymbal array. To measure interstitial glucose levels, the same cymbal array can also be used to permeabilize the skin. Combined with an electrochemical biosensor, the glucose concentration in the interstitial fluid can be determined after the skin becomes permeable using US delivered by the cymbal array. After the skin becomes permeable to glucose, the glucose diffuses through the skin and reacts with glucose oxidase in the hydrogel of the electrochemical biosensor, producing hydrogen peroxide. With amperometric biosensors, oxidation of hydrogen peroxide at the electrode surface generates an electrochemical current proportional to the concentration of glucose. Previous studies have shown the glucose sensitivity of the electrochemical biosensor consisting of the amperometric electrode and the glucose-oxidase containing hydrogel (Sirkar and Pishko 1998). To verify the safety of the US exposure, histologic evaluation of the rat skin was performed after exposure to US under various conditions. Overall, the purpose of this study was to demonstrate the feasibility of noninvasive transdermal blood glucose determination in rats, using the ultrasonic system that consists of the cymbal array and the electrochemical biosensor.

## MATERIALS AND METHODS

### Ultrasound array

Details regarding the design and construction of the cymbal transducer and the multielement array are described elsewhere (Lee et al. 2004; Newnham and Dogan 1998; Smith et al. 2003a). Briefly, the cymbal transducer is a class V flextensional transducer that uses very low frequency (in the kilohertz range) signals and possesses

a compact, lightweight structure with a resonance frequency adjustable in the range 1 to 100 kHz (Dogan et al. 1997; Newnham et al. 1994; Newnham and Dogan 1998; Tressler et al. 1998). The design of the cymbal transducer integrates two metal caps epoxyed onto a lead zirconate-titanate (PZT) ceramic. These caps create a shallow cavity between their inner surface and the ceramic (Fig. 1a). The fundamental mode of vibration is the flexing of the end cap caused by the radial motion of the ceramic; the overall displacement of the device is a combination of the axial motion of the disk ( $d_{33}$ ) and the radial motion amplified by the end cap ( $d_{31}$ ). For this research, the cymbal transducer consisted of a PZT-4 piezoelectric disk that has a diameter of 12.7 mm and a thickness of 1 mm (Piezokinetics, Inc., Bellefonte, PA, USA). The piezoelectric disk is bonded between two titanium metal caps, each of which has a thickness of 0.25 mm, using epoxy (Eccobond<sup>®</sup>, Emerson & Cuming, Billerica, MA, USA). To construct the array, four cymbal transducers were connected in parallel and encased in

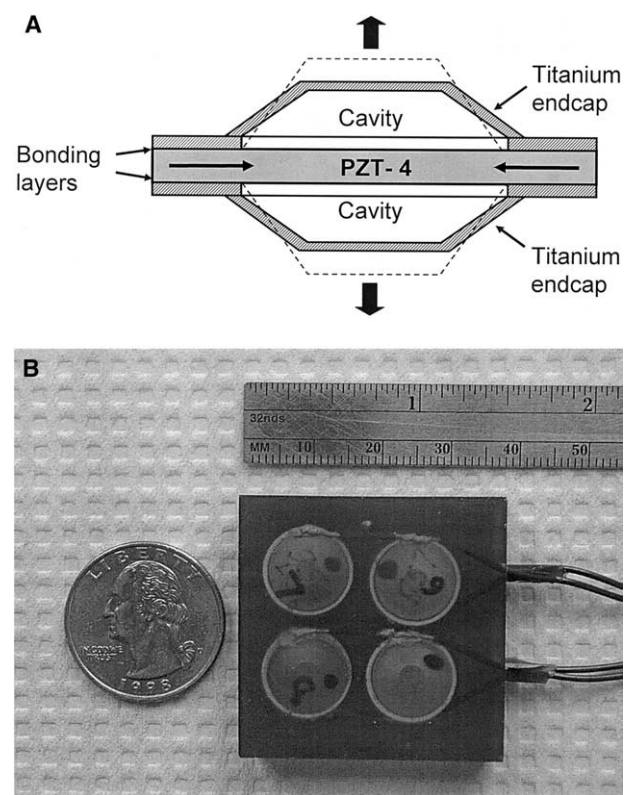


Fig. 1. (a) Cymbal disk made of piezoelectric material PZT-4 operated at a frequency of 20 kHz. Between two titanium caps, cymbal disk was placed with air cavities beneath the caps, which give rise to radial oscillations of the disk. (b) Lightweight low-profile array constructed using four cymbal transducers connected in parallel and encased in URALITE<sup>®</sup> polymer. After construction, dimensions of the array were 37 × 37 × 7 mm<sup>3</sup>; it weighed less than 22 g.

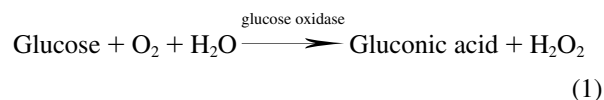
Uralite<sup>®</sup> polymer (FH 3550, H.B. Fuller, St. Paul, MN, USA). The four-element array was arranged in a two-by-two pattern and encased within a  $37 \times 37 \times 7 \text{ mm}^3$  polymer block (Fig. 1b).

To drive the array, a radio-frequency (RF) signal was generated by a frequency pulse/function generator (model 393, Wavetek Inc., San Diego, CA, USA) and amplified by an RF amplifier (model 40A12, Amplifier Research, Souderton, PA, USA). The electrical impedance of each transducer was tuned to the output impedance of the amplifier by an external tuning network. Pulse period, duty cycle and exposure time of the RF signal from the frequency generator were monitored using an oscilloscope (2213A, Tektronix, Beaverton, OR, USA).

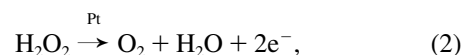
The pressure field of the cymbal array, which is used to calculate its intensity, was measured in a water tank ( $51 \times 54 \times 122 \text{ cm}^3$ ) with a calibrated miniature (4-mm diameter) omnidirectional reference hydrophone (model TC4013, S/N: 5199093, Reson, Inc., Goleta, CA, USA). The tank, which was made almost anechoic using sound-absorbing material placed at its walls, was filled with degassed ( $< 1$  to  $2 \text{ ppm}$ ) distilled water. A hydrophone was placed 1 mm from the array face and positioned by a computer-controlled positioning system (Velmex Positioning System, Velmex Inc., East Bloomfield, NY, USA) to scan an area of  $51 \times 51 \text{ mm}^2$  parallel to the array face. The peak compressional ( $p_c$ ) and rarefactional ( $p_r$ ) pressures were acquired using an oscilloscope (54622A, Agilent, Palo Alto, CA, USA).  $I_{\text{SPTP}}$  and spatial-peak pulse-average intensity ( $I_{\text{SPPA}}$ ) were determined based on three to five repeated scans of the array for a mean and SD of the results. For the glucose-determining experiment, the signal generator was driven at 20 kHz with a peak-to-peak voltage of 600 mV, a pulse duration of 200 ms, and a pulse-repetition period of 1 s (*i.e.*, 20% duty cycle). With these parameters, the peak compressional ( $p_c$ ) and rarefactional ( $p_r$ ) pressures were 38500 and  $-38500 \text{ Pa}$ , respectively, and the resultant intensity,  $I_{\text{SPTP}}$ , was  $100 \text{ mW/cm}^2$ .

#### Background and fabrication of electrochemical biosensor

Generally, electrochemical biosensors produce an electrical signal proportional to changes of physiological parameters such as blood pH levels, hematocrit, the partial pressure of oxygen, calcium and glucose (Peura 1992). Specifically for glucose electrochemical biosensors, glucose reacts with oxygen ( $\text{O}_2$ ) and water ( $\text{H}_2\text{O}$ ) in the presence of glucose oxidase to produce gluconic acid and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), as shown:



In this reaction, one glucose molecule generates one hydrogen peroxide molecule, which is oxidized in the electrochemical biosensor. When a positive electrical potential is applied and a platinum (Pt) modified electrode is used, hydrogen peroxide is electro-oxidized to oxygen and water with two electrons ( $2e^-$ ),



The current produced at oxidizing potentials is proportional to the hydrogen peroxide concentration (Wilkins and Atanasov 1996). These reactions require aqueous environments where the glucose oxidase might be washed out without a proper immobilization procedure.

For this research, the electrochemical biosensors fabricated based on amperometric electrodes (Fig. 2) were acquired from DuPont<sup>®</sup> Electronic Technologies (Research Triangle Park, NC, USA). Platinum, a catalyst for the hydrogen peroxide that was generated by glucose oxidase, was deposited on the working electrode after the electrodes were received. To deposit platinum, 25 mM of platinumic acid (P5775, Sigma-Aldrich Corp., St. Louis, MO, USA) in 0.1 mol/l NaCl solution was placed over these three electrodes. Between the working electrode and reference electrode, a voltage of 0.5 V was applied for 1 h to deposit platinum using a potentiostat (model 283, Princeton Applied Research, Oak Ridge, TN, USA).

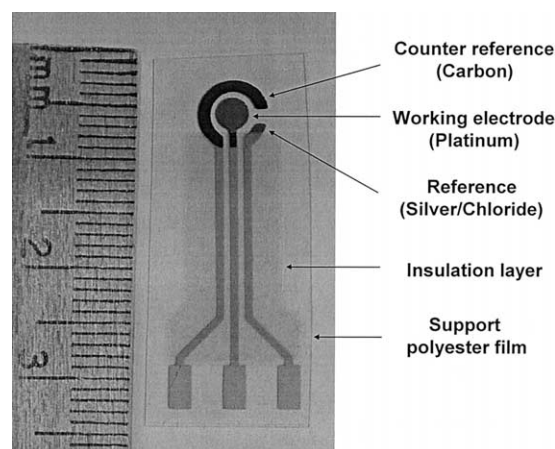


Fig. 2. Electrochemical biosensors fabricated based on polymer thick film (PTF) electrodes acquired from DuPont Electronic Technologies. Working electrode was carbon covered with platinum; reference electrode was Ag/AgCl; counter reference electrode was carbon.

The immobilization of glucose oxidase is necessary to keep the enzyme on the surface of the platinum electrode. Polyethylene glycol (PEG) hydrogel is a suitable material for an electrochemical biosensor, because the hydrogel swells considerably in the presence of water. The ions or the molecules can easily diffuse into the swelled hydrogel (Revzin et al. 2002; Russell et al. 2001). Diacrylated PEG (437441, MW 575, Sigma-Aldrich) was diluted with distilled water to make a 20% (v/v) solution and mixed with liquid photoinitiator (2-hydroxy-2-methyl-1-phenyl-1-propanone, Darocure 1173, Ciba Specialty Chemicals, Tarrytown, NY, USA) and glucose oxidase (500000 unit/g, G-7141, Sigma-Aldrich). Three electrodes on an electrochemical biosensor were covered with the solution of PEG, glucose oxidase and photoinitiator (30  $\mu$ l) and exposed to a light with 300 mW/cm<sup>2</sup> intensity and 365-nm wavelength for 2 s.

A concentration curve was prepared for each electrochemical biosensor to determine the unknown glucose concentration in interstitial fluid. A series of glucose solutions (0, 50, 100, 150, 200, 300, 400 and 500 mg/dl) were prepared by dissolving glucose (D-glucose, D9434, Sigma-Aldrich) in phosphate buffer solution (P3774, Sigma-Aldrich). The electric potential of the biosensor at each concentration was recorded before each experiment.

#### *In vivo ultrasound and glucose sensor experiments*

A total of 12 Sprague Dawley rats (300 to 400 g) were divided into two groups: 10 rats in the US exposure group and 2 rats in the control group. Each animal was anesthetized and euthanized by procedures approved by the Institutional Animal Care and Use Committee (IACUC) at the Pennsylvania State University. After the animal was anesthetized with a combination of ketamine hydrochloride (60 mg/kg IM, Ketaject<sup>®</sup>, Phoenix, St. Joseph, MO, USA) and sodium xylazine (10 mg/kg IM, Xyla-Ject<sup>®</sup>, Phoenix), the abdominal areas of the rats were shaved using an electric shaver and a depilatory agent was applied to the skin for 1 min to eliminate any remaining hair. With a rat placed in the dorsal decubitus position, a 1-mm thick water-tight stand-off was arranged between the abdomen and the array (Fig. 3a) and held in place using double-sided polyethylene adhesive tape (3 M, St. Paul, MN, USA). The reservoir inside the stand-off was filled with saline through small holes in the back of the array. Care was taken to remove all the bubbles from the solution in the reservoir between the abdomen and the array.

Before beginning the experiment, 0.3 ml of blood was collected from the jugular vein of each rat for a baseline glucose analysis with a glucose meter (ACCU-CHEK<sup>™</sup>, Roche Diagnostics, Indianapolis, IN, USA).

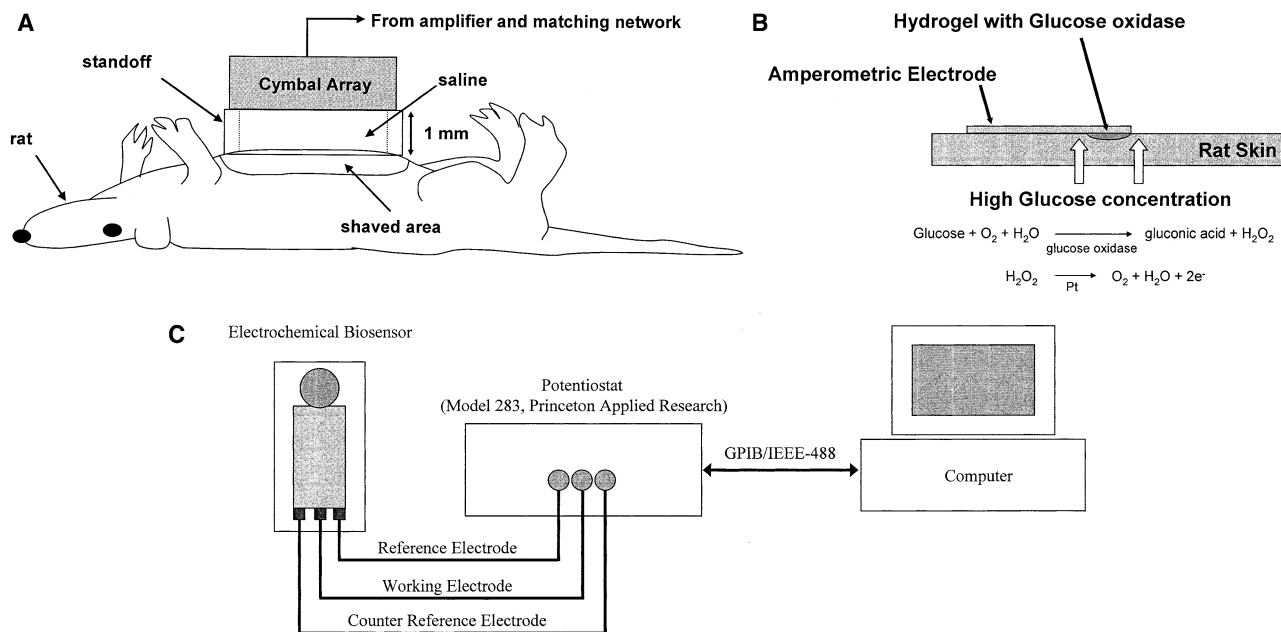


Fig. 3. (a) Rat on its back; 1-mm thick water-tight stand-off between the shaved abdomen and array. Reservoir inside the stand-off filled with saline. US ( $I_{SPTP} = 100 \text{ mW/cm}^2$ ) applied to exposed group for 20 min; US not applied to the control. (b) In presence of glucose oxidase, glucose becomes gluconic acid *via* glucono- $\delta$ -lactone: hydrogen peroxide is generated as byproduct. Electrochemical biosensor uses the signal caused by oxidation of hydrogen peroxide. One glucose molecule generates an oxygen molecule, a water molecule and two electrons in the reaction. (c) Voltage between working electrode and reference electrode, 0.7 V, applied from potentiostat controlled by computer. At the same time, current between two electrodes is determined by potentiostat and recorded in the computer.

Ultrasound ( $I_{\text{SPTP}} = 100 \text{ mW/cm}^2$ ) was applied to the US exposure group for 20 min; for the control group, a similar set-up was used without the array operating. After the array was removed, the electrochemical biosensor was placed on the center of the exposed skin (Fig. 3b). Simultaneously, the potentiostat (model 283, Princeton Applied Research, Oak Ridge, TN, USA) applied a voltage of 0.7 V to the working electrode against the reference electrode of the biosensor (Fig. 3c) and the current between the electrodes was recorded in the computer using the potentiostat control program (Electrochemistry PowerSuite™ v. 2.1.1, Princeton Applied Research). At the end of the experiment, the rats were euthanized under anesthesia.

For an accurate comparison between the conventional measurement and the ultrasonic biosensor method, blood glucose levels were measured with the glucose meter while the electrochemical biosensor was operating. The error of the electrochemical biosensor was estimated by calculating the mean difference between the biosensor and glucose meter values. Statistical analysis was performed using InStat® (GraphPad Software, San Diego, CA, USA) and the data of each group were analyzed as its mean and SD ( $\bar{x} \pm \text{SD}$ ). An ANOVA was used to analyze the statistical significance of the differences between the means of groups.

### Histology

A total of 7 Sprague Dawley rats (300 to 400 g) were used for histologic study to evaluate whether or not any microscopic lesions were produced by the intensity level ( $I_{\text{SPTP}} = 100 \text{ mW/cm}^2$ , 20% duty cycle) used in the previous glucose-sensor experiment. Intensity levels greater than the current experiment were also used specifically to cause damage. Each animal was anesthetized and the array was placed on the abdomen of the animal as previously described. Each rat was exposed to US for 20 min with different intensities or duty cycles listed in

Table 1. For the histology experiments, US exposure condition for each rat with the 20-min duration US

Group name	Intensity ( $I_{\text{SPTP}}$ , $\text{mW/cm}^2$ )	Duty cycle (%)
Control	0	NA
*100-20	100	20
200-20	200	20
300-20	300	20
100-15	100	15
200-15	200	15
300-15	300	15

Group 100-20 = the group exposed to US of  $100 \text{ mW/cm}^2$  intensity and 20% duty cycle; group 200-15 = group exposed to US of  $200 \text{ mW/cm}^2$  intensity and 15% duty cycle, and so on. Control = group exposed to no US. Two rats were used for each group.

\* The condition used for *in vivo* US and glucose sensor experiments.

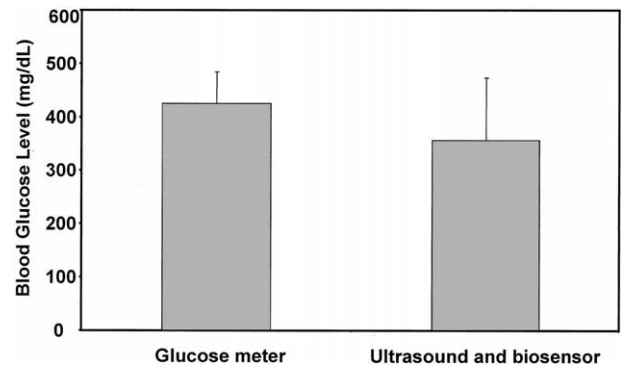


Fig. 4. Blood glucose level measured by standard glucose meter ( $424.8 \pm 59.1 \text{ mg/dl}$ ) and by electrochemical biosensor ( $356.0 \pm 116.6 \text{ mg/dl}$ ) after 20-min US exposure at  $100 \text{ mW/cm}^2$ . Comparison of the two groups indicated no statistically significant difference ( $p$  value  $> 0.05$ ).

Table 1. The first column indicates the name of each group and the second and third columns show the intensity and the duty cycle used for the group in the first column. For example, group 100-20 means that the group was exposed to US of  $100 \text{ mW/cm}^2$  intensity and 20% duty cycle. At the end of each experiment, the rat was euthanized with carbon dioxide while under anesthesia. The US-exposed full-thickness skin was removed from the animal, formalin-fixed, paraffin-embedded, cut in  $5\text{-}\mu\text{m}$  sections, stained with hematoxylin and eosin and examined by the Pennsylvania State University attending small animal veterinarian to evaluate cellular damage.

## RESULTS

### *In vivo* glucose sensor experiments

After the current between the working and the reference electrodes of the biosensor was determined, the glucose concentration in the interstitial fluid was obtained using the concentration curve previously made for each biosensor. The glucose levels from the biosensor and the standard glucose meter with US exposure are shown in Fig. 4. After 20 min of US exposure, blood glucose levels determined by the glucose meter and the electrochemical biosensor were  $424.8 \pm 59.1 \text{ mg/dl}$  and  $356.0 \pm 116.6 \text{ mg/dl}$ , respectively, with an error of  $68.8 \pm 130.0 \text{ mg/dl}$ . Without US exposure, the venous blood glucose level of the control group determined by the glucose meter was  $424.5 \pm 71.4 \text{ mg/dl}$ . The average measurement of the electrochemical biosensor without US exposure was only  $39.5 \pm 0.7 \text{ mg/dl}$ , giving rise to an error of  $386 \pm 70.7 \text{ mg/dl}$ . To determine the statistical significance between the results, an ANOVA was used to analyze the data. The  $p$  value result for the comparison between the blood glucose levels determined by the

glucose meter ( $424.8 \pm 59.1$  mg/dl) and the electrochemical biosensor ( $356.0 \pm 116.6$  mg/dl) was larger than 0.05, which indicated that there was no statistical difference.

### Histology

Based on gross and light microscopic evaluation of skin after US exposure, gross and histologic lesions were only observed in groups 200-20 and 300-20 (Fig. 5d). Lesions were localized and appeared to correspond to the

area directly under one of the transducers. No significant lesions were observed in the control or other treatment groups, including the level of US exposure used for the glucose sensor experiments.

With an intensity of  $300 \text{ mW/cm}^2$  and a duty cycle of 20% (group 300-20), a large area ( $1.5 \text{ mm}$  across  $\times$   $1.5 \text{ mm}$  deep) of tissue destruction extended through the dermis, the subcutaneous tissue and underlying superficial musculature. Lesions included degeneration of collagen, destruction of hair follicles and sebaceous glands,

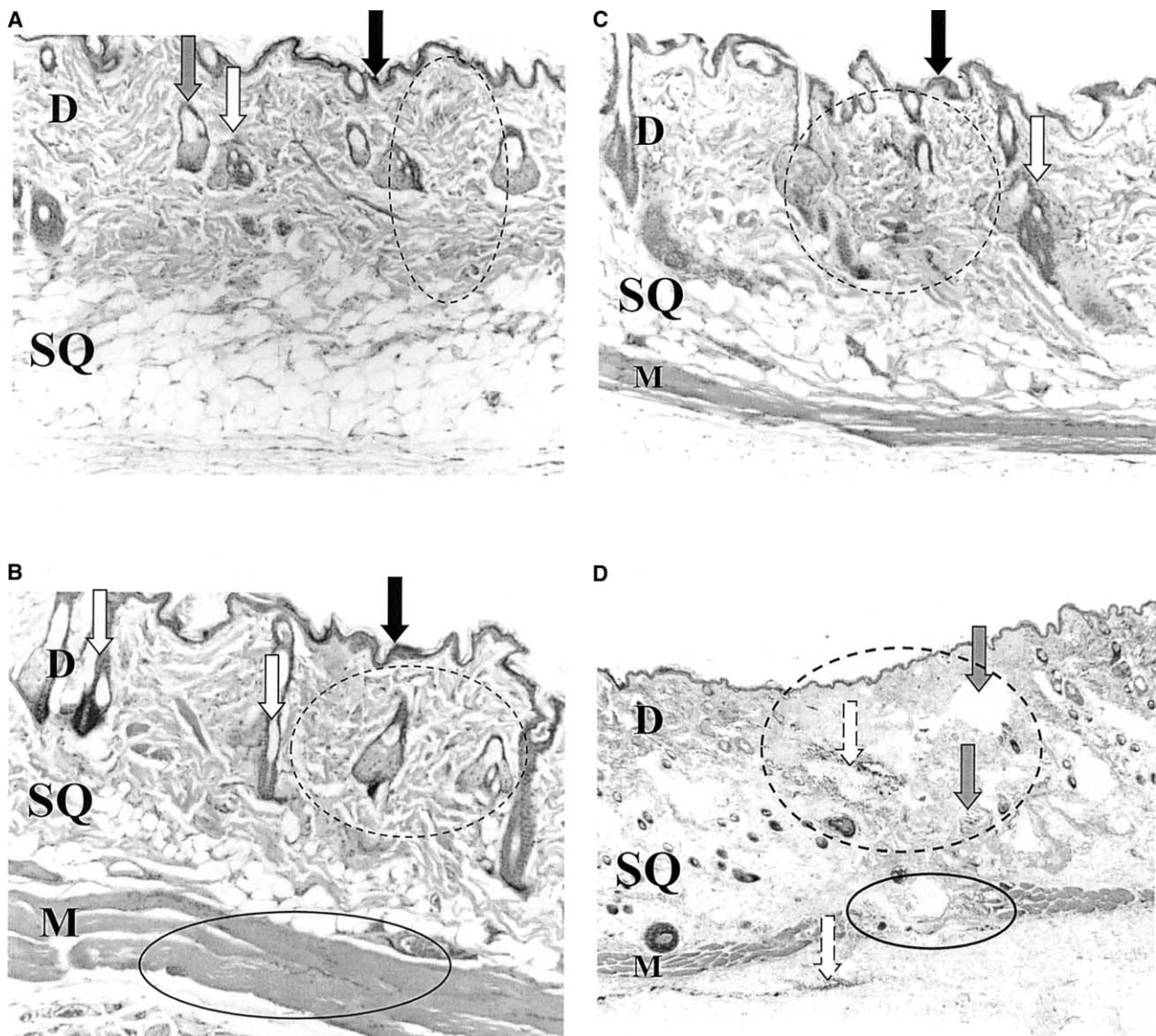


Fig. 5. Hematoxylin and eosin-stained sections of skin representing: (a) Control: no US exposure; (b)  $100 \text{ mW/cm}^2$ , 20% duty cycle (group 100-20); (c)  $300 \text{ mW/cm}^2$ , 15% duty cycle (group 300-15); and (d)  $300 \text{ mW/cm}^2$ , 20% duty cycle (group 300-20). D = dermis; SQ = subcutaneous tissue; M = muscle; black arrow = epidermis; white arrows = hair follicles; gray arrows = sebaceous glands; (---) an area of the dermis; (—) cutaneous musculature. No significant lesions observed in (a), (b) or (c). In (d) collagen destruction (notice poor definition and granular appearance of collagen in dermis; compare with control), areas of hemorrhage (dashed white arrows), destruction and loss of hair follicles and sebaceous glands, and necrosis of cutaneous musculature are observed.

disruption of subcutaneous adipose tissue, muscle degeneration and multiple areas of hemorrhage. Large open areas or cavities were present within the lesion and the surrounding subcutaneous tissue, suggesting subcutaneous fluid accumulation.

A large area (0.75 mm across  $\times$  0.6 mm deep) of tissue destruction was also observed in group 200-20 (200 mW/cm<sup>2</sup>, 20% duty cycle). However, an artefact of tissue preparation precluded evaluation of the epidermis and dermis of the affected area. Lesions observed were similar, but less severe than those observed in group 300-20 and included degeneration of collagen, disruption of hair follicle and sebaceous gland architecture and large open spaces or cavitated areas.

## DISCUSSION

The glucose measurement results using the electrochemical glucose biosensor after US exposure have shown a reliable accuracy compared with conventional measurement using the glucose meter. The apparent error of the electrochemical biosensor was around 69 mg/dl. Without US, however, the biosensor could not detect the glucose level and the apparent error of the biosensor was 386 mg/dl in the hyperglycemic rats (425 mg/dl, based on the measurement of the glucose meter). The results from the control group showed that, without US exposure, glucose could not be diffused through the rat skin, despite shaving and applying the depilatory agent. The efficacy of a portable glucose-management system based on an electrochemical biosensor combined with the cymbal array has been demonstrated by the *in vivo* glucose sensor experiments.

The safety and the potential bioeffects of the US were evaluated at the various US exposure conditions. Histology results showed that the condition of the US used in noninvasive blood glucose measurement, I<sub>SPTP</sub> = 100 mW/cm<sup>2</sup> and 20% duty cycle, did not generate any significant damage. However, with the higher intensity, 200 and 300 mW/cm<sup>2</sup> at 20% duty cycle, destruction of skin tissue was observed, including hemorrhage and destruction of the epidermis and collagen. It can be inferred from the results that even 2 or 3 times the intensity will not generate any significant bioeffect if the duty cycle is kept at 15% or less.

In conclusion, US-mediated noninvasive glucose measurements have been performed using the electrochemical glucose sensor with the cymbal array. In previous studies, insulin delivery has been enhanced by utilizing US both *in vitro* and *in vivo*. Combined with a noninvasive transdermal insulin delivery system, this research will develop a feedback system for a portable

blood glucose management device based on the cymbal array.

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