

Transdermal monitoring of glucose and other analytes using ultrasound

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Considerable effort has been directed towards developing painless and convenient methods to measure blood analytes, particularly glucose¹, including implantable sensors²⁻⁴, minimally invasive skin microporation approaches involving laser or miniaturized lancets⁵ and noninvasive technologies such as near-infrared spectroscopy⁵, transdermal permeation enhancers⁶ or reverse iontophoresis^{7,8}. However, none are used in routine clinical practice⁵. One of the fundamental problems in noninvasive transdermal diagnostics is obtaining sufficient quantities of analyte for detection. Ultrasound, particularly at low frequencies, enhances transdermal delivery of drugs (sonophoresis)⁹⁻¹¹. Here, we sought to determine whether such ultrasound facilitates the outward transport of analytes present in the interstitial fluid, thereby allowing noninvasive extraction of clinically useful analytes¹².

Transdermal analyte extraction *in vitro* and *in vivo*

We first studied *in vitro* permeability using human cadaver skin. We assessed the transdermal transport of three representative model analytes (glucose, urea and calcium) as well as a model drug (theophylline) using low-frequency ultrasound. Ultrasound application led to *in vitro* transdermal extraction of all molecules (Fig. 1a), with permeabilities several orders of magnitude higher than those obtained with passive diffusion across skin. For example, passive skin permeability of glucose was about 0.0003 cm/h, compared with 0.17 cm/h after ultrasound application (an increase of 570-fold). We used control experiments to ensure that the extracted analytes originated from the donor compartment and not skin itself; in these, the donor

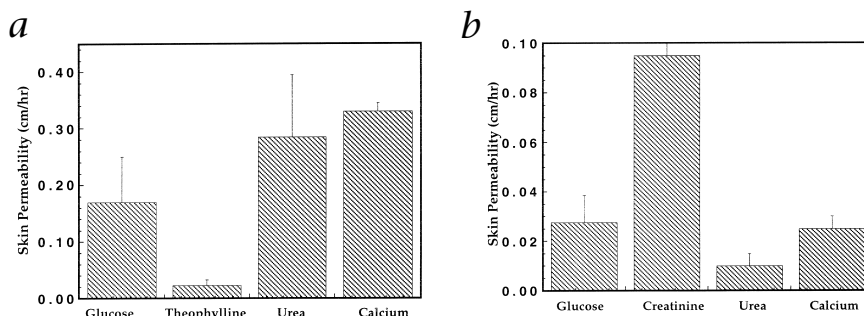
compartment was filled with phosphate-buffered saline (PBS) instead of serum, followed by ultrasound extraction. There were no substantial amounts of analytes, including glucose (less than 0.04%), urea (less than 0.3%) or calcium (less than 1%), in the receiver compartment after 10 minutes of ultrasound exposure.

We used Sprague Dawley rats in initial *in vivo* studies to assess the efficacy of ultrasound to extract the molecules across living skin. We noted extraction of glucose, calcium, urea and creatinine after ultrasound application (Fig. 1b), with permeabilities substantially more than those obtained with passive diffusion. For example, glucose permeability, which was undetectable without treatment, increased to 0.026 ± 0.011 cm/h after ultrasound irradiation. We also evaluated the importance to the enhanced transport of each step in the protocol, that is, hydration, skin permeabilization using ultrasound and extraction using ultrasound (or vacuum). Skin permeability to glucose was undetectable before hydration, 0.0004 ± 0.0003 cm/h immediately after hydration, 0.0034 ± 0.0025 cm/h immediately after ultrasonic skin permeabilization and 0.026 ± 0.011 cm/h during ultrasonic extraction (or 0.034 ± 0.025 during vacuum-based extraction). We measured the time variation of these permeabilities over a period of 3 hours using twelve extractions (15 minutes each). The measured skin permeability remained within 10%, indicating that skin permeability of a given site was steadily maintained over a considerable amount of time.

Correlation of extracted glucose with blood glucose

To determine if the amount of glucose extracted represented a meaningful physiologic determination of venous blood

Fig. 1 Ultrasound-enhanced skin permeability. **a**, Sonophoretic permeability of analytes across human skin *in vitro*. The final concentrations of the analytes in the collection chamber at the end of the 10-minute extraction period were 0.11 mM for glucose (1.98 mg/dl), 4250 d.p.m./ml for theophylline, 0.11 mM for urea, and 0.22 mg/dl for calcium. Skin permeability, P , and chamber analyte concentration, C , are related by the equation: $C = PC, At/V$, where C_s is the serum analyte concentration, A is the chamber area (1.7 cm^2), t is the time of extraction, and V is the receiver fluid volume (2 ml). **b**, Sonophoretic permeability of analytes across rat skin *in vivo*. The final concentrations of the analytes in the collection chamber at the end of the 15-minute



extraction period were 0.073 mM (1.3 mg/dl) for glucose, 0.017 mg/dl for creatinine, 0.061 mg/dl for urea, and 0.059 mg/dl for calcium.

Methods

In vitro transport experiments. The *in vitro* permeability experiments used human cadaver skin (obtained from local hospitals and the National Disease Research Institute). The skin was heat-stripped by keeping full-thickness skin in water at 60 °C for 2 min, followed by removal of the epidermis, which was then stored at 4 °C in a humidified chamber for up to 2 weeks and mounted on a Franz diffusion cell (FDC 400; Crown Glass, Somerville, New Jersey). The epidermis was supported by a nylon mesh (Sefar, Depew, New York) to avoid damage due to possible mechanical oscillations upon ultrasound application. The receiver compartment was filled with PBS (0.01 M phosphate and 0.137 M NaCl; Sigma), except for calcium transport experiments, in which it was filled with deionized water. Neither PBS nor the deionized water used in these experiments was degassed. Before each experiment, the electrical resistance of the skin was measured to ensure its integrity. (The skin was considered damaged if the initial specific skin resistance was less than 10 k Ω -cm².) The donor solution was filled with calf serum (Sigma). Typical concentrations of analytes in the serum were 80 mg/dl glucose, 16 mg/dl urea and 5 mg/dl calcium. ³H-theophylline was added to the donor compartment at a concentration of 0.5 μ Ci/ml. Experiments were also completed using ¹⁴C-labeled glucose. In those experiments, the donor compartment was filled with 0.5 μ Ci/ml solution of ¹⁴C glucose (NEN) in PBS. Ultrasound was applied using a sonicator (VCX 400, Sonics and Materials, Newtown, Connecticut) operating at a frequency of 20 kHz. The sonicator consists of a signal generator (about 6 × 12 × 6 inches) and a horn (a tapered cylinder about 10 inches long and 3 inches in diameter at its widest end). The displacement of the tip of the horn was adjusted to 2 μ m (following the manufacturer's specifications). Ultrasound was applied continuously for 10 min. Ultrasound intensity was also measured using a calorimetric method¹⁶. Analytes were extracted for 10 min while the ultrasound was on.

The concentration of glucose in the diffusion cell compartments was detected using two methods. When ¹⁴C-labeled glucose was used, the concentration was measured by a scintillation counter (Packard model 2000, Meriden, Connecticut). The concentrations of unlabeled glucose, urea and calcium were measured using spectrophotometric detection kits (315 for glucose, 586 for calcium and 535 for urea, all from Sigma). The reagent-

to-sample ratio in these kits was modified to a value of 2 for glucose and calcium, and 10 for urea, to lower the concentration detection limit. Theophylline concentration in the diffusion cell compartments was measured using a scintillation counter (Packard).

In vivo transport experiments. All animal procedures followed institution-approved protocols. Rats (Sprague Dawley, either sex) were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally or intramuscularly. Injection of xylazine increased blood glucose values. After sedation was confirmed, a flanged glass cylinder (diameter, 15 mm; height, 2 cm; Crown Glass,) was glued onto the rat's shaved lateral flank using a minimal amount of cyanoacrylate adhesive (Permabond International, Inglewood, New Jersey or Vet Bond, Roanoke, Virginia). The chamber was hydrated with PBS or deionized water for 1 h. At the end of the 1-hour hydration, ultrasound (20 kHz, 11 μ m (based on manufacturer's specifications), pulsed 5 s on and 5 s off) was applied by immersing the transducer in the donor solution for 2 min. After 2 min, the contents were removed and replaced (after being rinsed) with fresh buffer (2 ml PBS). Ultrasound was again applied at a lower intensity (tip displacement, 1 μ m.) for an additional 15 min, after which the chamber content was replaced by fresh PBS (the removed volume was used for analyte detection). Low-intensity ultrasound application was repeated 12 times (total length of experiments, 3 h). At the end of the experiment, the rat was killed and the skin exposed to ultrasound was excised and stored in 10% formalin for histologic studies. Skin samples were stained with hematoxylin and eosin and viewed with a microscope.

In some experiments (Fig. 2a), after sedation was confirmed, the jugular vein was catheterized as described¹⁷. Ultrasound (tip displacement of 11 μ m) was applied for 2 min to permeabilize skin. An intravenous injection of 200 μ Ci radiolabeled glucose was given immediately after the 2 min of ultrasound exposure. Fifteen-minute extractions were done as described above using low-intensity ultrasound. A second injection of 200 μ Ci radiolabeled glucose was given 85 min after the first injection. In tracking experiments (Fig. 2b), animals were infused with a solution of insulin (10 mU/min) through the jugular vein to vary the blood glucose level. Extractions were done as described above. A calibration factor between serum glucose level

glucose, we injected rats through the jugular vein with two boluses of ¹⁴C-radiolabeled glucose, 85 minutes apart. The curves for the noninvasively extracted glucose flux followed the blood glucose concentrations (Fig. 2a). An efficient way to compare relationships between transdermal flux and blood glucose values is to compare the blood glucose values predicted based on transdermal flux and those directly measured using blood samples (Fig. 2b). We obtained a good correlation for both hypoglycemic and hyperglycemic ranges (Fig. 2b; $r = 0.93$). Another criterion for accuracy is the mean relative error between the reference and calculated glucose value, defined by: relative error = [absolute(serum glucose - calculated glucose)/serum glucose] × 100. We obtained a mean relative error of 15% for all measurements ($n = 45$), a value similar to that of self-testing devices now

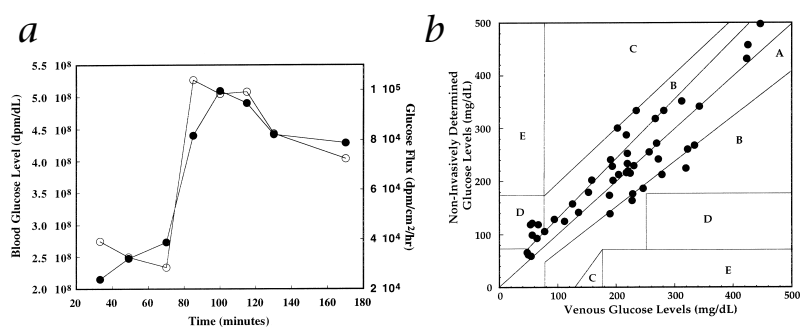
available¹³. We analyzed histologically rat skin samples exposed to multiple ultrasound applications in conditions identical to those used for glucose extraction. There was no physical damage in any of the histologic specimens. Furthermore, the regions of rat epidermis exposed to ultrasound were intact and showed no abnormalities.

Clinical data

We next assessed this technique on humans to determine whether a single, short application of ultrasound was sufficient to extract glucose noninvasively across human skin for several hours, and to determine whether transdermal glucose flux varied in response to variations in blood glucose levels induced by a standard meal of Sustacal (Fig. 4a, protocol). We recruited

Fig. 2 Glucose extraction and correlation in rats.

a, Correlation (*in vivo*) of noninvasive determination of glucose with blood glucose measurements: ○, blood glucose level (d.p.m./dl); ●, glucose flux. **b**, Error grid analysis dividing a plot comparing the reference serum glucose and the measured glucose (calculated from the measured noninvasive glucose flux) into five zones. Values in zones A and B are clinically acceptable¹³. The average glucose concentration in the chamber when the rat had a blood glucose level of 100 mg/dl was 0.022 mM. The mean relative error in predictions is 13%. The standard error of prediction SEP = square root (SEP = square root of {average (calculated glucose - serum glucose)²}) is 40 mg/dl. Values of SEP averaged separately for blood glucose levels in the range



of 0–100 mg/dl, 100–200 mg/dl and >200 mg/dl are 27 mg/dl, 30 mg/dl and 42 mg/dl, respectively.

and transdermal flux was calculated by dividing the first glucose flux by the second serum glucose level (lag time of one extraction). This factor was then used to calculate serum glucose from transdermal flux for all subsequent measurements. The calibration factors (expressed as [mg/dl]/[nmol/cm²/h]) for five rats were 0.38, 0.68, 1.37, 0.62 and 1.4. Only one site was used per rat in our experiments. Thus, the variation of calibration factor within different sites on the same animal cannot be obtained.

Clinical experiments. Seven volunteers (four males and three females 18–40 years of age) participated in this study. The volunteers had been diagnosed as having type 1 diabetes mellitus for between 1 and 20 years, without peripheral vascular disease or other known diabetic complications. Written informed consent was obtained from all volunteers. The protocol was approved by the institutional review boards of Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center. Patients were admitted to the clinical research center and instructed to follow their usual daily meal and insulin injection regimen. A glass chamber (about 1.5 cm in diameter) was placed on the forearm, secured in place with an elastic strap, and filled with 1 ml sterile saline for 1 h. An indwelling catheter was introduced in the other forearm for removing blood samples for repeated blood glucose analysis.

At the end of 1 h, the saline was removed from the chamber and filled with 2 ml of 1% sodium lauryl sulfate in saline. Ultrasound (20 kHz; 11- μ m tip displacement; pulsed 5 s on/5 s off) was applied to the chamber. The ultrasound transducer (1 cm in diameter) was placed at a distance of 1 cm from the skin and was activated for up to 2 min. In therapeutic ultrasound, the maximum allowed intensities are about 2 W/cm² for a typical application of 15 min (corresponding to a total energy dose of 1,800 J/cm²)¹⁸. In our experiments, typical ultrasound intensities were 10 W/cm² (spatial average temporal peak intensity, which typically corresponds to a displacement of 11 μ m as measured by calorimetry¹⁶) applied to the skin for 2 min at a duty cycle (the percent of time when ultrasound is on) of 50%. This application resulted in a total energy dose of 600 J/cm². The total acoustic power applied to the skin was about 13 W. Ultrasound was applied only once for less than 2 min before the 4 h of intermittent vacuum extractions.

After ultrasound application was completed, the sodium lauryl sulfate

solution was then replaced by 0.5 ml saline. Vacuum (10 inches Hg) was applied to the chamber for 5 min. At the end of vacuum application, the solution in the chamber was removed for glucose measurement and the chamber was refilled with 2 ml saline, and covered with parafilm until the next vacuum application. Vacuum applications of 5 min were repeated twice every 30 min. Thus, samples corresponding to a total of nine different time points were collected in duplicate. Samples that were subject to experimental error (due to leakage) were excluded from the analysis (3 of a total of 53 samples).

The samples from the chamber taken during vacuum applications were assayed for glucose (Catalog no. 315; Sigma). Blood samples (2.5 ml) were obtained from the venous catheter to measure blood glucose concentration. A meal of 240 cc Sustacal was given 1 h after the ultrasound application to increase blood glucose levels. The sampling procedure was repeated for an additional 3 h. Minor 'chamber pressure imprint' was noted on the skin at the end of the experiments caused by the attached chamber and vacuum application. This was completely resolved within a few hours. The absence of any discernible untoward effect of ultrasound is relevant, because many transdermal patches in wide clinical use (for example, estradiol) cause severe skin irritation¹⁹. At the end of the experiment, patients were interviewed about the comfort of sonophoretic analyte monitoring. The first four patients returned after 2 d to have the skin exposed to ultrasound re-evaluated; subsequent patients were interviewed by phone.

The calibration of transdermal flux and serum glucose is similar to that used in animal experiments; that is, the first glucose flux was used to calculate the calibration factor and a time lag of one extraction (30 min) between flux and serum glucose was allowed for each. Thus, the calibration factor was calculated by dividing the first glucose extraction flux by the second blood glucose value. These calibration factors (in the units of [mg/dl]/[nmol/cm²/h]) for seven patients were 2.2, 7.4, 1.3, 6.6, 3.8, 0.7 and 2.3. Although the calibration factor varied about 10-fold between different patients, it remained steady at a given site on the patient. The measured variation in the calibration factor (originating from the variation in skin permeability) within a given site on a patient was less than 20% over a period of 4 h.

seven patients with Type 1 diabetes (free of diabetic complications), after obtaining approval by the institutional review boards. We measured skin permeability on all seven patients at a single site (mid-volar forearm) after ultrasound application. The average skin permeability varied from person to person (from 0.0013 ± 0.00096 cm/h to 0.013 ± 0.0014 cm/h). However, the time variation of these permeabilities at a given

site for a given patient over a period of 4 hours was within 20% (a standard deviation commonly obtained with existing glucose measurement methods¹⁴). We did additional experiments to further assess the duration of ultrasound-induced permeability. The skin permeability remained high for about 15 hours and decreased to its normal value by 24 hours (Fig. 3a). The average transdermal glucose fluxes after ultrasound application are 25–

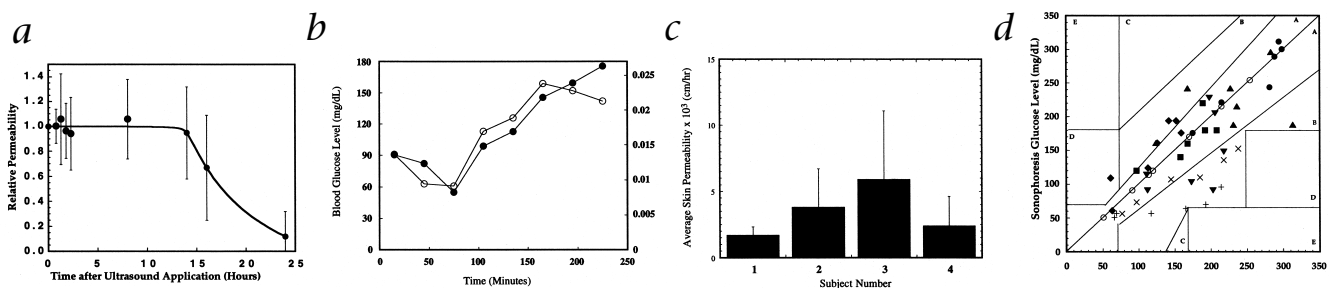
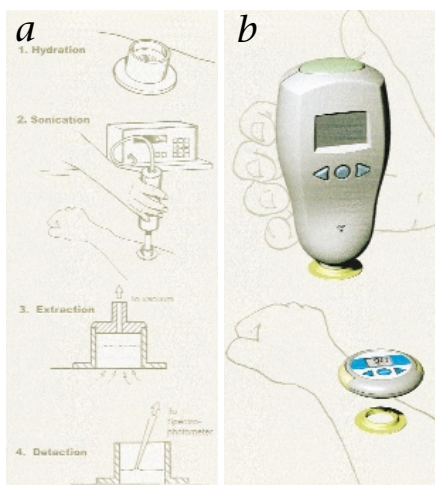


Fig. 3 Glucose extraction and correlation in humans. **a**, Skin permeability recovery after ultrasound application, in four healthy volunteers (2–3 sites on each) and seven type 1 diabetic volunteers (single site on each). Data represent average (\pm s.d.) on all sites on all subjects. **b**, Blood glucose levels (\circ) compared with noninvasive glucose flux (\bullet), over time. **c**, Site-to-site variability within the same subject and subject-to-subject variability. Different locations ($n = 3$ –5) between the elbow and the wrist on volar and dorsal forearms of four healthy human volunteers were tested. Data represent average \pm s.d. of all sites on each subject. **d**, Error grid for all volunteers based on one-point calibration point. The actual blood glucose levels used for calibration are different for different patients (range,

50–250 mg/dl for seven subjects). Data represent a mean absolute error of 20%. Average glucose concentration in the receiving chamber at the end of 5-minute extraction was 0.015 mM when the subject had a blood glucose level of 100 mg/dl. The mean relative error between the calculated glucose levels and the reference glucose level is 23%. The standard error in predictions is 52 mg/dl (SEP values for each of seven subjects (in mg/dl) were 17, 22, 31, 63, 62, 61 and 70). The SEP values averaged separately (over all subjects) for blood glucose levels in the range of 0–100 mg/dl, 100–200 mg/dl and >200 mg/dl are 22 mg/dl, 51 mg/dl and 62 mg/dl, respectively. Each symbol (except for \circ) corresponds to extractions on the same subject; \circ , points used for calibration.

Fig. 4 Current protocols and product concept vision. **a**, Clinical protocol: 1, 1 h of hydration; 2, short ultrasound application; 3, 5 min of vacuum-based extraction; 4, detection of extracted glucose. **b**, Product that could be developed based on the technology described here, consisting of a hand-held and battery-operated ultrasound device to permeabilize the skin, and a patch (to be placed on ultrasonically permeabilized skin) for continuous extraction, detection and show of glucose levels. The hydration step in **a** is absent here, based on our data indicating that similar transdermal glucose fluxes can be obtained *in vitro* and *in vivo* even when the hydration step is omitted (further research is necessary to confirm this result in human volunteers).



fold higher than the fluxes reported for the reverse iontophoresis technique⁷. High fluxes of analytes are important in enabling readily detectable analyte concentrations, short collecting times, small skin area required for sampling and low interference from background analytes (for example, sweat).

A comparison of venous blood glucose levels and noninvasively extracted glucose fluxes showed close correlation (Fig. 3b). The extracted glucose flux profile was shifted to a later time relative to the serum profile, indicating a lag time of one sample period. The extracted glucose may be coming from capillaries under the surface of the skin located at the junction of the dermis and epidermis. Therefore, the lag time between blood glucose measurements and ultrasound-extracted glucose might have been related to the diffusion of capillary glucose across the epidermis and stratum corneum. We also evaluated the site-to-site variability (within same patient) and patient-to-patient variability (averaged over a number of sites) of skin permeability. The site-to-site variability (Fig. 3c, standard deviations on each bar) was about the same as patient-to-patient variability (Fig. 3c, variations in the average values of bars). This indicates the necessity of one-point calibration between transdermal glucose flux and one blood sample, which can then be used to predict subsequent blood glucose values. Based on such a calibration, we assessed the relationship between transdermal glucose flux and blood glucose values using an error grid similar to that in Fig. 2b (Fig. 3d). Predictions in zones A and B are clinically acceptable, whereas those in zones C, D, or E would lead to clinically significant errors¹³. All but one of the 53 predictions based on transdermal glucose flux were in zones A and B (Fig. 3d).

Patients reported no pain during ultrasound application and we could detect no visible effect of the ultrasound on the skin. Although further studies assessing safety (especially the effect of repeated extractions) will be required, our results indicate that with the conditions we used, low-frequency ultrasound does not seem to induce damage to skin or underlying tissues.

The possibility of using ultrasound to enhance transdermal transport of diverse substances of wide-ranging molecular size

and chemical composition could be useful in both diagnostics and drug delivery. The results are especially encouraging given that the ultrasound device used in this study (Sonics and Materials VCX400) was not designed or optimized for this application. After further research on the portability, stability and long-term safety of ultrasonic devices, a hand-held, battery-operated device might be developed to permeabilize the skin in combination with a patch (Fig. 4b) or a wristwatch¹⁵ displaying glucose levels continuously for the day or night. Such a device could be used at home or in a clinician's office for intermittent (used once a day) or continuous (showing trends and having alarms for hypoglycemic events) monitoring. This approach could be further developed to combine the sensing and delivery parts into one design (wristwatch or patch). This would allow the design of closed-loop transdermal systems in which the delivery of drug (for example, insulin)⁹ is modulated in response to physiological requirements.

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