Analytical Characterization of Electrochemical Biosensor Test Strips for Measurement of Glucose in Low-Volume Interstitial Fluid Samples

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Background: **Minimally invasive interstitial fluid (ISF) sampling and glucose measurement technologies were integrated into a hand-held device for diabetic glucose monitoring investigations.**

Methods: **Conventional electrochemical test strip technology (Bayer Glucometer Elite®) was adapted to measure glucose in small (0.5–2.0 μL) samples of ISF. Test strip glucose measurements were performed on a commercial potentiostat and were compared to various reference glucose methodologies (YSI 2300 analyzer, microhexokinase procedure, Bayer Glucometer Elite). Characterizations of the integrated ISF sampling-glucose test strip design included accuracy and precision in various sample media (saline, ISF surrogates, diabetic ISF samples), sample volume dependence, test strip sterilization studies (electron beam, γ irradiation), and diabetic ISF sampling and glucose measurements.**

Results: **Glucose measurements were free from significant media effects. Sample volume variations (0.6–3.2 μL) revealed only modest dependence of glucose measurement bias on sample volume (−1.5% per microliter). Sterilization treatments had only a minor impact on glucose response and test strip aging and no significant impact on interferent responses of the glucose test strips. Diabetic subject testing under minimum fasting conditions of at least 2 h with integrated ISF sampling and glucose measurement gave low ISF glucose measurement imprecision (CV, 4%) and mean glucose results that were indistinguishable from reference (microhexokinase) ISF glucose measurements and from capillary blood glucose measurements (Glucometer Elite).**

Conclusions: **Conventional single-use, electrochemical glucose test strip and ISF collection technologies can be readily integrated to provide real-time ISF sampling and glucose measurements for diabetic monitoring applications.**

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Measurement of ambient glycemia is of critical importance to people with diabetes mellitus. This was highlighted by the results of Diabetes Control and Complications Trial *(1)* published in 1993, which concluded that intensive therapy in conjunction with aggressive monitoring of blood glucose concentrations can lead to better control of the disease, thus slowing the progression of long-term microvascular and neurologic complications. Currently, people with diabetes who self-monitor their blood glucose do so by lancing their fingertip to obtain a sample of blood. This sample is then transferred to a measurement strip for glucose analysis by a hand-held meter. Unfortunately, lancing of the fingertip to obtain blood samples is frequently painful, messy, and inconvenient.

To reduce the pain, mess, and inconvenience associated with glucose monitoring, many minimally and noninvasive glucose measurement systems are currently being developed. These technologies include near-infrared radiation transmission through, or reflectance from, body tissue; reverse iontophoretic or chemically enhanced fluid extraction from the skin; use of alternative body fluids; light scatter from body tissues; photoacoustic spectroscopy; Raman spectroscopy; and ocular fluid polarization changes. These techniques have been reviewed extensively *(2–5)* and show varying degrees of promise.

In pursuit of a less invasive glucose measurement system, Integ has developed a proprietary method for extracting fluid from the dermis. This sampling method involves penetration by a cannula into the dermis and

allows for the routine collection of ~1 μL of interstitial fluid (ISF), with a median collection time of between 4 and 5 s. Studies have suggested that glucose in ISF samples collected via this method closely reflects ambient glycemia (6) and that there is no clinical difference between glucose in the interstitial and venous compartments in subjects whose glucose concentrations are changing rapidly (7). These studies concluded that a correlation exists between ISF glucose and venous plasma glucose.

Many techniques have been used to sample and characterize fluid collected from the cutaneous and subcutaneous layers of skin. For example, blister fluid has been collected and studied as an analog for ISF. Studies conducted on human subjects concluded that blister fluid total protein is one-third that of serum and that there is a highly significant correlation between skin glucose and plasma glucose (8–10). Additional research utilizing suction effusion fluid and subcutaneous tissue fluid collected by the liquid paraffin cavity method and the wick method have shown that the total protein concentration in tissue fluid is approximately one-third that of plasma with an increased albumin/globulin ratio relative to plasma (11–16). Work done with subcutaneous implanted glucose sensors has shown that ISF glucose correlates well with plasma glucose (17–19). Several studies with microdialysis probes have concluded that the glucose concentration in the subcutaneous tissue is equivalent to venous blood glucose under steady-state conditions (20–24). However, one of these studies also tested subjects after a very rapid increase in blood glucose and found a significant difference between ISF glucose and blood glucose concentrations (21). Research performed in our laboratory has shown that the ISF collected by the Integ sampling technology is consistent with the foregoing literature characterizations of ISF in that the total protein concentration is approximately one-third that of plasma and the albumin/globulin ratio is, on average, 1.85.

We reported previously on an earlier attempt to integrate our ISF sampling technology with mid-infrared measurement technology in a hand-held ISF glucose meter (25). In a correlation study of 445 subjects with type 1 and type 2 diabetes under 2-h fasting conditions, a standard error of prediction of 241 mg/L (24.1 mg/dL) and correlation coefficient of 0.95 was obtained using the infrared methodology when compared to venous plasma glucose.

Here, we report our efforts to integrate the ISF collection technique with an electrochemical biosensor measurement technology similar to measurement technologies typically used in conventional self-monitoring blood glucose meters. The objectives of these studies were to (a) demonstrate the feasibility of integrating ISF collection with conventional, single-use, electrochemical test strip glucose measurement technology and (b) to characterize the glucose measurement performance of an integrated ISF collection and glucose test strip device. Glucose measurement characterizations included studies of dependence on sample medium and sample volume, sterilization treatment, interferent response, and precision and accuracy.

Materials and Methods

INTEGRATION OF ISF COLLECTION AND GLUCOSE TEST STRIP MEASUREMENTS

ISF sample collection and glucose measurement were integrated through use of an adapter that mates a conventional, single-use, electrochemical glucose test strip with a 30-gauge needle assembly (Fig. 1). This integration permits direct sampling of ISF from the dermis and real-time sample transfer to the test strip read area for subsequent ISF glucose measurement. All measurements made with disposable integrated ISF collection-glucose test strips were performed in single-use mode.

GLUCOSE TEST STRIP

The glucose test strip was a modified, electrochemical strip (Glucometer Elite®; Bayer, Diagnostics Division) that uses potentiostatically controlled working and counter electrodes in combination with glucose oxidase enzyme and ferricyanide electron transfer mediator reagents (26). The glucose test strips were physically modified to allow coupling of the test strip to the ISF collection adapter. To preserve glucose test strip activity, the integrated ISF collection-test strip assemblies were stored over desiccant and protected from light before use. Because of the requirement for sterile ISF collection needles, test strips were also subjected to sterilization treatments to model the glucose measurement performance that could be expected from sterilized, integrated ISF collection-test strip assemblies.

ISF COLLECTION, SAMPLE DETECTION, AND GLUCOSE MEASUREMENT

The integrated ISF collection-test strip assemblies required sample volume of 0.5–1.0 μL to perform glucose
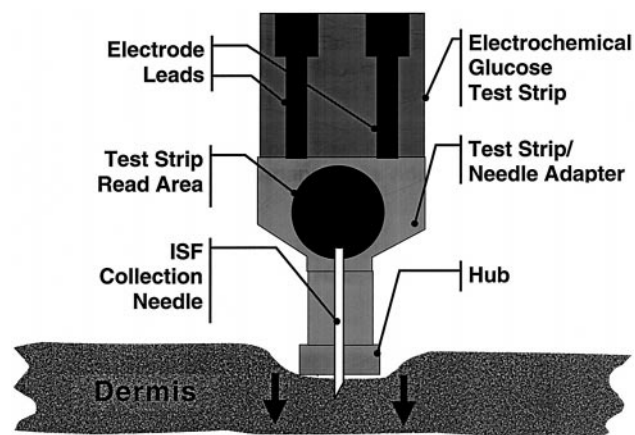
Fig. 1. Schematic of integrated ISF collection and electrochemical test strip device for direct sampling of ISF and real-time measurement of ISF glucose.

measurements. In use, the integrated ISF collection-test strip assembly was housed in a hand-held ISF sampling device that provided rapid collection of ISF from the forearm. The ISF sampling device was tethered to a custom sample-detect circuit that detected placement of the sampler on the arm. Additionally, the circuit detected ISF wetting of the test strip as a decrease in resistance between the test strip electrodes. Detection of sampler placement on the arm and ISF wetting of the test strip enabled calculation of the time required for ISF collection with each measurement. The sample-detect circuit provided an audible prompt for the user to remove the sampler from their arm. The circuit then connected the test strip electrode leads to a potentiostat control circuit for subsequent measurement of sample glucose. Two potentiostat control circuits were used. An unmodified Glucometer Elite meter was used in selected studies to perform glucose measurements with the integrated ISF collection-test strip assemblies. Glucometer Elite meter readings were adjusted by an appropriate calibration response curve to correct for the impact of the ISF collection adapter and the ISF surrogate media on test strip glucose signals.

For all other studies, a commercial EG&G Princeton Applied Research model 273A potentiostat (EG&G Instruments) was used to perform the same test strip control functions as a conventional electrochemical glucometer circuit. The potentiostat was programmed with a 25-s delay to allow adequate glucose reaction time after sample detect. After the glucose reaction time was complete, the potentiostat was operated in a chronoamperometric mode for 10 s with the center working electrode of the test strip polarized at +0.50 V vs the outer counter electrode. During this polarization time, the potentiostat recorded the current flowing between the test strip electrodes. Current readings were made from the resulting current-time traces by averaging 0.5 s of current measurements centered around 5 s of polarization time (hereafter referred to as 5-s current readings). All glucose concentration predictions from EG&G potentiostat measurements were based on glucose calibration curves determined in physiologic saline media. Laboratory temperature was controlled (typically ±1.5 °C) so that compensation of glucose concentration predictions based on temperature was not necessary.

For ISF collection and glucose measurement studies, informed consent was obtained from diabetic subjects enrolled under a WIRB®-approved protocol for integrated ISF sampling and glucose measurement. Other studies required delivery of ISF surrogate samples to the integrated ISF collection-test strip assemblies. For some studies, surrogate sample was delivered directly to the test strip read area by a digital hand pipetter (Pipetman models P2 and P10; Rainin Instrument). In other studies, surrogate sample was delivered to the test strip through the ISF collection needle by a syringe pump (KdScientific model 200; KD Scientific) and Hamilton glass syringe

(25-μL model 720RN; Hamilton) connected to the ISF collection needle via plastic tubing.

REAGENTS AND SAMPLE MEDIA

Nonsterile isotonic saline reagent (9.0 g/L NaCl) was from Fisher Scientific. D-(+)-glucose, human albumin (fraction V), human α-globulins (Cohn fraction IV-1), Triton X-100® surfactant (*t*-octylphenoxypolyethoxyethanol), and sodium phosphate (dibasic, anhydrous) were obtained from Sigma. Chemicals for interference testing included acetaminophen (Tylenol®), uric acid, L-ascorbic acid (vitamin C), L-(+)-lactic acid, acetylsalicylic acid (aspirin), bilirubin (mixed isomers), cholesterol, and triglycerides [triolein, $C_{18:1}$, (cis)-9], all obtained from Sigma. ProClin 150 preservative was from Supelco.

Physiologic glucose concentrations were prepared in various media by adding microliter volumes of a 1 mol/L glucose stock solution prepared in deionized water (MilliQ Plus deionizer; Millipore). Sample media included physiologic saline, synthetic ISF surrogates, and human plasma- and serum-based ISF surrogates. Synthetic ISF surrogates were prepared by dissolving human albumin and α-globulins in a ratio of 60 g/L albumin to 40 g/L α-glob ulins in isotonic saline containing 6 mg/L ProClin 150 preservative to achieve total measured protein concentrations of 11 and 31 g/L (1.1 and 3.1 g/dL). Human plasma-based surrogates were prepared from human plasma isolated by centrifugation of whole blood drawn into 7-mL gray-stoppered Vacutainer® Tubes (14 mg of potassium oxalate and 17.5 mg of sodium fluoride; Becton Dickinson). Plasma aliquots were pooled and diluted 1:2 (500 μL + 500 μL) with isotonic saline to give 1:2 diluted plasma-based ISF surrogate. We assumed the total protein concentrations of 1:2 diluted plasma-based ISF surrogate to be 35 g/L (3.5 g/dL). Human serum-based surrogates were prepared from human serum isolated by clotting and centrifugation of whole blood drawn into 7-mL red-stoppered Vacutainer Tubes. Serum aliquots were pooled and diluted 1:3 (333 μL + 667 μL) with 60 mmol/L phosphate-buffered isotonic saline, pH 7.4, to give 1:3 diluted serum-based ISF surrogate. Buffered diluent was used for serum-based ISF surrogates to buffer the surrogate against addition of acidic interferent test compounds (see below). We assumed the total protein concentration of 1:3 diluted serum-based ISF surrogate to be 23 g/L (2.3 g/dL). Assumed protein concentrations were used for plasma- and serum-based ISF surrogates because synthetic surrogate studies demonstrated negligible impact of protein concentration variations [0–31 g/L (0–3.1 g/dL)] on ISF glucose measurements (see *Results and Discussion*). Saline glucose calibrators and synthetic and human plasma- and serum-based ISF surrogate solutions were stored at −70 °C.

GLUCOSE REFERENCE MEASUREMENTS

Reference glucose concentration measurements were made in ISF surrogate samples using a YSI model 2300

Stat Plus glucose analyzer (Yellow Springs Instruments). The YSI glucose measurements differed from direct, undiluted test strip measurements as a function of the protein content of the sample because of the sample dilution used in the YSI 2300. Accordingly, ISF surrogate reference glucose measurements were corrected for YSI sample dilution errors by dividing the YSI result by the water content of the sample $\{1 - [\text{protein concentration } (g/dL)]/100\}$ (27). For synthetic ISF surrogates, total protein concentrations measured by electrophoresis were used for YSI dilution corrections. For plasma- and serum-based ISF surrogates, YSI dilution corrections assumed 35 g/L (3.5 g/dL) protein for 1:2 diluted plasma surrogates and 23 g/L (2.3 g/dL) protein for 1:3 diluted serum surrogates.

Reference glucose concentrations were measured in diabetic ISF samples by a an enzymatic hexokinase method modified for use with microliter sample volumes (hereafter called microhexokinase procedure). For this procedure, ISF samples of ~1.5 μL were collected into 2-μL heparin-containing capillary tubes (Drummond Scientific) and were stored at $-70\,^{\circ}$C. Modifications to the standard single-reagent, manual hexokinase procedure (kit no. 16-50; Sigma Diagnostics) were necessary because of the small ISF sample size, the range of volumes collected, and the spectral sensitivity requirements at low glucose concentrations. The standard procedure described by Sigma requires a 10.0-μL sample volume and 1.00-mL reagent volume. The procedure was modified to accommodate volumes of between 0.50 and 2.00 μL and a reagent volume of 75.0 μL. To account for the variable sample volumes, each ISF sample was weighed using an MT5 microbalance (Mettler-Toledo), and a gravimetric correction was applied. Absorbances were measured with a Spectronic Genesys 5 spectrophotometer (Milton Roy).

GLUCOSE TEST STRIP STERILIZATION AND INTERFERENT STUDIES

The impact of sterilization on the glucose response, aging, and selectivity of the electrochemical glucose test strips was modeled by exposing test strips in their original packaging to γ-irradiation (Steris-Isomedix) and electron-beam (Titan Scan Systems) sterilization treatments at 20 and 40 kGy doses. The 20-kGy dose represents the radiation dose required to sterilize the needle assembly shown in Fig. 1. Test strip aging time points were 0, 1, and 4 months of real-time aging (ambient storage temperature, 20–25 °C) and accelerated equivalent aging of 6 months [storage for 36 days at 45 °C in an ESPEC environmental chamber (model LHU-112; ESPEC)]. Test strips were stored unopened in their original packaging for all sterilization and aging treatments (0% relative humidity was assumed for the package headspace).

The glucose responses of sterilized and nonsterilized test strips were determined for each experimental treatment by measuring 10 replicates at each of two glucose concentrations (5 and 15 mmol/L) in synthetic ISF surrogate medium [31 g/L (3.1 g/dL) total protein; 5-μL sample volume]. Aside from the sterilization and aging treatments, the test strips used in this study were unmodified (i.e., test strips were not combined with ISF collection needle adapters). All glucose measurements were performed on a single unmodified Glucometer Elite meter according to the manufacturer's instructions.

The effect of the sterilization treatment on test strip glucose selectivity was determined by measuring the response of glucose test strips to various endogenous and exogenous compounds that have potential to interfere with electrochemical glucose measurements $(28–30)$. The sample medium used for interference testing was 1:3 diluted human serum-based ISF surrogate containing 4.7 mmol/L glucose. Serum-based ISF surrogate was used for interference studies to avoid contaminating the sample medium with the anticoagulants and antiglycolytic preservatives present in plasma collection tubes. The glucose concentration for the interference studies was based on NCCLS testing guidelines (31) and was chosen to represent the lower acceptable limit for controlling blood glucose as established by a survey of physicians (32) Interferent concentrations were chosen based on NCCLS testing guidelines (31). Most interferent test samples were prepared by adding interferent stock solution to serum-based ISF surrogate. Because of solubility limitations, triglyceride and cholesterol interferent test samples were prepared as emulsions by direct dispersion into ISF surrogate containing 5 g/L Triton X-100 surfactant. ISF surrogate pH extremes (pH 6.8 and 8.8) were prepared by adding microliter amounts of concentrated HCl or NaOH. Appropriate precautions were taken to avoid exposure of interferent test solutions to air and light.

Interferent responses were measured on nonsterilized and sterilized (electron beam, 20-kGy dose) glucose test strips. Unmodified, nonsterilized control test strips were tested for interferent responses, using 5.0-μL sample volumes on an unmodified Glucometer Elite meter. Sterilized test strips were integrated with ISF collection needle adapters (Fig. 1). These were subsequently tested for interferent responses using 2.0-μL sample volumes delivered by pipet to the test strip read area followed by measurement of chronoamperometric current-time response curves using the EG&G potentiostat configuration outlined above. Seven replicates were tested for each interferent test solution on both nonsterilized and sterilized test strips, and glucose measurement biases were calculated relative to a control sample to which blank, buffered saline had been added.

Results and Discussion

SAMPLE MEDIUM AND GLUCOSE RESPONSE

The glucose calibration response of the integrated ISF collection-electrochemical glucose test strip device (Fig. 1) was measured in physiologic saline medium. Glucose samples (0.5 μL) covering the concentration range 1.9–29 mmol/L (35–529 mg/dL; six concentrations; five repli-
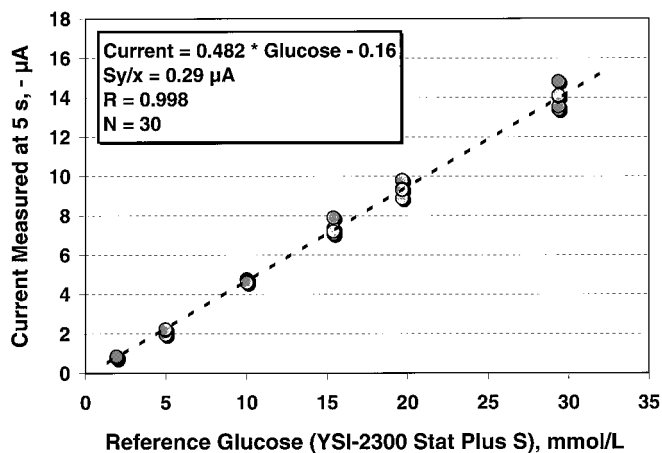
Fig. 2. Glucose calibration response of the integrated ISF collection and glucose measurement disposable in physiologic saline medium.

cates per concentration) were pipetted into the ISF collection-test strip assemblies, and the glucose current vs time response curves were recorded using the EG&G potentiostat. Glucose current readings were plotted against the corresponding YSI reference glucose concentrations to produce the glucose calibration response curve for saline medium (Fig. 2). The saline calibration response was linear from 1.9 to 29 mmol/L with a correlation coefficient of 0.998, an $S_{y|x}$ of 0.29 μA, and an intercept statistically indistinguishable from zero [95% confidence interval (CI) = −0.35 to 0.04 μA; Table 1].

Glucose measurements were also performed in 0.5-μL samples of synthetic and human plasma-based ISF surrogates [1.9–32.6 mmol/L glucose (35–586 mg/dL); six concentrations; five replicates per concentration]. Glucose current measurements were made in each of the ISF surrogate media, and corresponding medium-specific glucose calibration curves and regression statistics were generated (Table 1). All media exhibited calibration intercept 95% CIs that were statistically indistinguishable from zero. Comparison of calibration slope 95% CIs revealed slight statistical differences between the sample media, with the protein-containing ISF surrogate media consistently exhibiting slightly lower slopes than the saline media [largest slope difference, 7.5%, saline vs 31 g/L (3.1 g/dL) synthetic surrogate].

To determine the clinical significance of the small slope differences observed between the glucose response curves in different media, additional measurements were made in each sample medium at 5 and 20 mmol/L (90 and 360 mg/dL) glucose. Glucose current readings in each medium were used to make glucose concentration predictions from the saline calibration curve of Fig. 2. At 5 mmol/L glucose (Table 2), the mean relative glucose prediction biases were similar between the different ISF surrogate media (range, −1.9% to 2.0%). The saline medium exhibits a slightly higher mean relative glucose prediction bias (8.2%), suggesting slight positive curvature in the saline glucose response curve near 5 mmol/L. With exclusion of a single data point from the 31 g/L (3.1 g/dL) synthetic ISF surrogate data set, all media exhibited CVs of 5% or less at 5 mmol/L glucose. Similar results were observed for 20 mmol/L glucose (Table 3), with mean relative glucose prediction biases ranging from 1.6% to 3.6% and glucose prediction CVs ranging from 1.6% to 3.6%. For all media, the 5 and 20 mmol/L glucose prediction biases were well within the Clarke error grid A zone (±20% bias) designated as clinically accurate *(33)*. Thus, medium-dependent differences in glucose response appear to be clinically insignificant in terms of glucose prediction accuracy and precision.

SAMPLE VOLUME AND GLUCOSE RESPONSE

Physiologic and sampling variables produced a range of ISF sample volumes during ISF collection and measurement studies. The effects of sample volume variations on glucose prediction bias were studied by using a syringe pump to deliver synthetic ISF surrogate samples [31 g/L (3.1 g/dL) protein, 5 or 15 mmol/L glucose] through the needle of the ISF collection-test strip assembly. Variations in sample volume delivered to the test strip read area were produced by varying syringe pump sample delivery rates (2, 8, or 20 μL/min) and by introducing variable delays (0, 1.5, or 3 s) before terminating sample delivery following sample detect. These operating variables were chosen to produce delivered sample volumes that ranged from 0.6 to 3.2 μL. Delivered sample volumes were recorded from the syringe pump after each measurement cycle. Glucose measurements were made using an unmodified Glucometer Elite meter connected to the elec-

Table 1. Comparison of glucose calibration regression statistics for different sample media.

Sample medium	n	Conc. range, mmol/L	Slope (95% CI), $\frac{\mu A}{mmol/L}$	Intercept (95% CI), μA	r	$S_{y|x}$, μA
Physiologic saline	30	1.9–29.4	0.482 (0.470–0.494)	−0.16(−0.35 to 0.04)	0.998	0.29
Synthetic ISF surrogate (11 g/L protein)	29	2.1–30.0	0.461 (0.453–0.469)	−0.06(−0.19 to 0.07)	0.999	0.19
Synthetic ISF surrogate (31 g/L protein)	29	2.2–30.4	0.446 (0.439–0.453)	0.04(−0.08 to 0.16)	0.999	0.18
1:2 diluted plasma ISF surrogate (35 g/L protein assumed)	30	2.5–32.6	0.464 (0.458–0.470)	0.03(−0.08 to 0.14)	1.000	0.16

Table 2. Comparison of within-run glucose prediction precision and accuracy for nominal 5 mmol/L glucose in different sample media.[a]

Sample medium	Reference glucose, mmol/L	Mean predicted glucose, mmol/L	SD predicted glucose, mmol/L	CV predicted glucose, %	Mean prediction bias, mmol/L	Mean relative prediction bias, %
Physiologic saline	4.9	5.3	0.25	4.7	0.4	8.2
Synthetic ISF surrogate (11 g/L protein)	5.2	5.1	0.10	2.0	−0.1	−1.9
Synthetic ISF surrogate[b] (31 g/L protein)	4.9	5.0	0.42	8.4	0.1	2.0
1:2 diluted plasma ISF surrogate (35 g/L protein assumed)	4.9	5.0	0.05	1.0	0.1	2.0
Mean			0.21	4.0	0.13	2.6
SD			0.17	3.3	0.21	4.2

[a] All glucose predictions are from the saline calibration of Fig. 2 (n = 15 replicates per medium).
[b] Exclusion of a single data point (6.3 mmol/L) gives predicted glucose mean of 4.9 mmol/L; SD, 0.11 mmol/L; CV, 2.3%; bias, 0.0 mmol/L and 0.0%.

trode leads of the integrated ISF collection-test strip assembly. Glucose measurement biases were calculated vs the appropriate YSI reference glucose measurements. Over the range of sample volumes studied (0.6–3.2 μL), the dependence of glucose prediction biases on sample volume was slight and on the order of −1.5% bias per microliter of increased sample volume (Fig. 3). ISF collection and sampling experience with the integrated ISF collection-test strip design suggested 0.9–2.5 μL as the worst-case range of sample volumes that can be expected from diabetic ISF sampling. Over this range of sample volumes, the impact of volume variations on glucose measurement bias is expected to be clinically insignificant.

STERILIZATION AND GLUCOSE RESPONSE
Current commercial home use glucose monitoring systems do not require sterilization of glucose test strips. However, integration of glucose measurement with biological fluid sampling needles may require sterilization of glucose test strips. Fig. 4 shows the effect of sterilization treatments on the glucose response and aging of the glucose test strips when tested with 5 mmol/L (90 mg/ dL) glucose samples. The initial impact of sterilization treatments is seen from the day 1 data in Fig. 4. Both γ-irradiation and electron-beam sterilization treatments initially increased glucose responses compared with the control by 10–30%, depending on the sterilization dose and method. At a given dose, sterilization-induced biases were higher for γ irradiation than for electron-beam sterilization. The more pronounced effect of sterilization by γ irradiation may be attributable to the greater oxidative reactivity expected from γ irradiation for a given sterilization dose (34). Sterilization-induced biases also increased with sterilization dose by both methods.

Inspection of the 1-, 4-, and 6-month (accelerated) stability time points in Fig. 4 reveals that sterilization-induced biases did not change dramatically over the course of the study. Sterilization-induced biases were smaller but exhibited similar patterns when tested at 15 mmol/L glucose, and sterilization and aging treatments had no appreciable impact on the precision of glucose measurements at either glucose concentration (data not shown). These results suggest that the impact of sterilization treatment on test strip glucose response may readily

Table 3. Comparison of within-run glucose prediction precision and accuracy for nominal 20 mmol/L glucose in different sample media.[a]

Sample medium	Reference glucose, mmol/L	Mean predicted glucose, mmol/L	SD predicted glucose, mmol/L	CV predicted glucose, %	Mean prediction bias, mmol/L	Mean relative prediction bias, %
Physiologic saline	19.5	20.2	0.67	3.3	0.7	3.6
Synthetic ISF surrogate (11 g/L protein)	18.7	19.3	0.30	1.6	0.6	3.2
Synthetic ISF surrogate (31 g/L protein)	18.6	18.9	0.34	1.8	0.3	1.6
1:2 diluted plasma ISF surrogate (35 g/L protein assumed)	19.6	20.2	0.41	2.0	0.6	3.1
Mean			0.43	2.2	0.6	2.9
SD			0.17	0.8	0.2	0.9

[a] All glucose predictions are from the saline calibration of Fig. 2 (n = 15 replicates per medium).
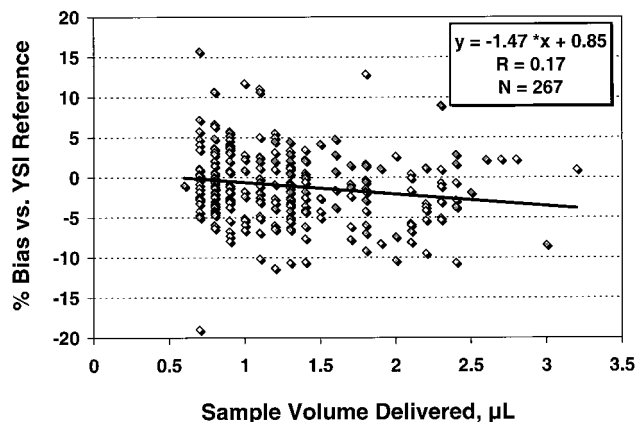
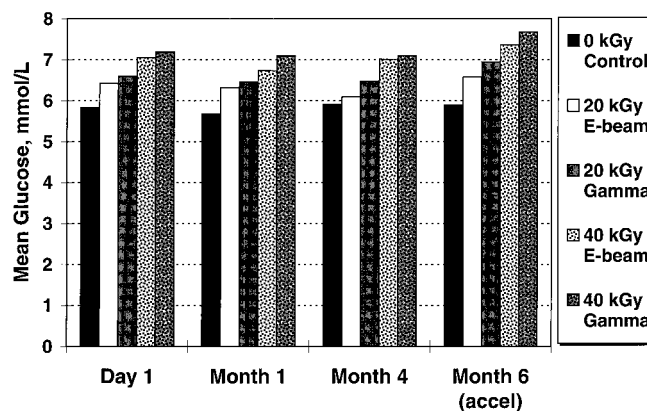Fig. 3. Impact of sample volume variations on glucose measurement biases.

Fig. 4. Effect of sterilization treatments on the glucose response and aging of electrochemical glucose test strips.

Nominal glucose concentration, 5 mmol/L.

be accounted for by appropriate sterilization process controls and strip calibration strategies. In addition, Table 4 shows that 20-kGy electron-beam sterilization treatment has no significant effect on the interferent response of the electrochemical glucose test strip. The results of these studies suggest that sterilized integration of glucose measurement and ISF sampling technology may be readily achieved with conventional electrochemical glucose test strip designs.

DIABETIC ISF GLUCOSE MEASUREMENT

Integrated ISF sampling-glucose measurement was studied in diabetic subjects after a minimum of 2 h fasting. The first of these studies focused on the accuracy of real-time electrochemical measurements of glucose in ISF samples collected using the integrated ISF collection-test strip assembly shown in Fig. 1. In this study (Fig. 5), pairs of capillary blood Glucometer Elite measurements were al-

ternated with sets of four ISF glucose measurements. The first set of four ISF measurements was made real-time on integrated, sterilized test-strip ISF collection adapter assemblies using the EG&G potentiostat. Reference micro-hexokinase glucose measurements were performed on the second set of ISF samples ($\sim$1.5 μL of ISF collected into each of four capillary tubes). The data shown in Fig. 5 are representative of results obtained from diabetic subjects and illustrate the good agreement between the two ISF glucose measurement methods as well as between ISF and capillary blood glucose measurements.

A second experiment focused on ISF glucose measurement precision during real-time integrated ISF sampling and measurement in diabetic subjects. For this experiment (Fig. 6), capillary blood glucose measurements on a Glucometer Elite were alternated with two ISF glucose measurements performed on the EG&G potentiostat for peri-

Table 4. The effect of 20-kGy electron-beam sterilization on the interferent response of the electrochemical glucose test strip.[a]

		Nonsterilized test strips			Sterilized test strips		
Interferent	Interferent concentration, mg/L	Mean glucose bias, mmol/L	95% CI	Mean relative bias, %	Mean glucose bias, mmol/L	95% CI	Mean relative bias, %
Acetaminophen	200	2.18	2.03–2.34	43.9	2.17	1.99–2.34	47.3
Uric acid	200	2.55	2.40–2.70	51.2	1.77	1.05–2.49	38.6
Ascorbic acid	30	0.21	0.08–0.33	4.0	0.29	0.02–0.56	6.2
Lactic acid	1000	0.09	0.03–0.15	1.8	−0.02	−0.37 to 0.33	−0.5
Acetylsalicylic acid	500	0.18	−0.04 to 0.39	3.6	−0.01	−0.29 to 0.28	−0.2
Bilirubin	200	0.00	−0.15 to 0.15	0.1	0.25	0.12–0.38	5.5
Cholesterol[b]	5000	0.18	−0.06 to 0.42	3.9	0.44	0.14–0.74	10.1
Triglycerides[b]	30000	0.17	−0.01 to 0.45	3.5	0.10	−0.28 to 0.48	2.4
Triton X-100, 5 g/L	5000	−0.26	−0.39 to −0.13	−5.2	−0.27	−0.52 to −0.01	−5.8
pH 6.8		0.18	−0.04 to 0.41	3.7	0.00	−0.23 to 0.23	0.0
pH 8.8		0.10	0.03 –0.16	2.0	−0.06	−0.19 to 0.07	−1.4

[a] Sample medium: 1:3 diluted human plasma, 4.7 mmol/L glucose. Glucose measurement biases were calculated relative to a control sample with added blank, buffered saline.

[b] Added 5 g/L Triton X-100 surfactant to aid dispersion of interferent in test solution; reported biases corrected for Triton X-100 bias.
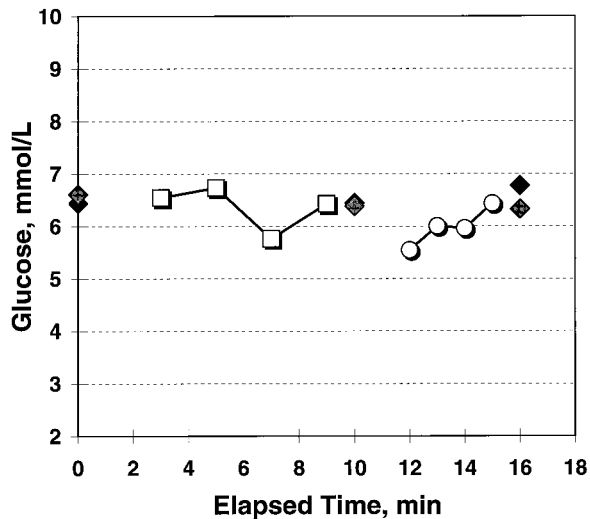
Fig. 5. Integrated ISF sampling and real-time electrochemical glucose measurements vs microhexokinase reference ISF glucose measurements and capillary blood glucose measurements on a diabetic subject (minimum fasting conditions, 2 h).

□, ISF, real-time electrochemical measurement; ○, ISF, microhexokinase measurement; ◆, capillary blood, Glucometer Elite, sample 1; ◇, capillary blood, Glucometer Elite, sample 2. Mean glucose results: 6.38 mmol/L for integrated ISF sampling + test strip (95% CI, 5.68–7.09 mmol/L); 5.99 mmol/L for microhexokinase (95% CI, 5.38–6.61 mmol/L); 6.50 mmol/L for capillary blood (95% CI, 6.28–6.71 mmol/L).

ods of 40–60 min for each subject. As can be seen from the representative subject results shown in Fig. 6, the ISF glucose measurements showed good agreement with capillary blood glucose measurements. Moreover, the precision of real-time, integrated ISF sampling and electrochemical glucose measurements compared favorably with the capillary blood glucose measurement precision. The observed ISF precision reveals both good glucose mea-
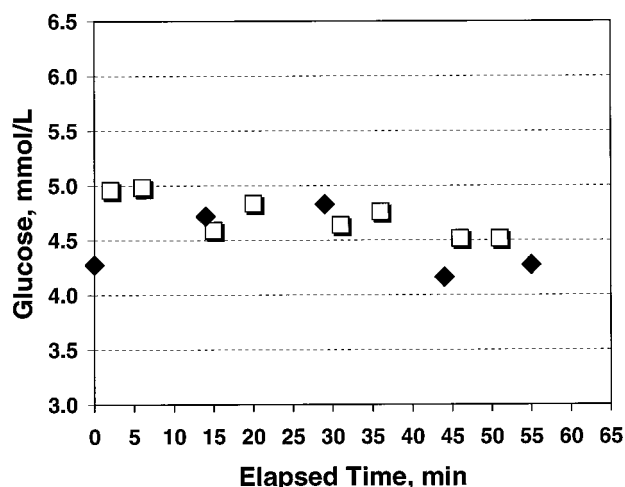

Fig. 6. Integrated ISF sampling and real-time electrochemical glucose measurements (□) vs capillary blood glucose measurements (◆) on a diabetic subject (minimum fasting conditions, 2 h).

Glucose results: for integrated ISF sampling + test strip, mean, 4.72 mmol/L (95% CI, 4.56–4.88 mmol/L), CV = 4.0%, n = 8; for capillary blood, mean = 4.45 mmol/L (95% CI, 4.08–4.82 mmol/L), CV = 6.7%, n = 5.

surement precision and low site-to-site variation of glucose in ISF samples. This minimal site-to-site sampling variability in ISF glucose concentrations is consistent with more detailed studies of site-to-site ISF sampling glucose variability conducted in our laboratories and communicated elsewhere in this issue (35).

In conclusion, this work has demonstrated that conventional single-use, electrochemical glucose test strip technology can be readily integrated with an ISF collection device to provide real-time ISF sampling and glucose measurements. This integration was accomplished with low ISF sample volume requirements and negligible sample volume dependence. In addition, the integrated ISF collection-glucose test strip assembly exhibited clinically insignificant dependence on sample medium and protein content and was only minimally affected by sterilization treatments. Lastly, diabetic studies of real-time ISF collection and glucose measurement gave good precision and accuracy. Together, these findings highlight the potential clinical utility of real-time, integrated ISF collection and electrochemical glucose measurements for diabetic glucose monitoring applications. Because ISF can be obtained in a substantially painless and blood-free operation, it is reasonable to expect that integration of accurate and precise glucose measurements with convenient ISF sampling would encourage aggressive monitoring of ambient glycemia among patients with diabetes mellitus. Indeed, subjects with diabetes who have participated in these tests have expressed a strong preference for the Integ nonfingerstick sampling methodology. Accordingly, we believe that integrated ISF sampling and glucose measurement technology will lead to improved glycemic control among patients with diabetes and ultimately to reductions in the long-term complications that result from diabetes (1).

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