Review

Progress Toward an In Vivo Surface-Enhanced Raman Spectroscopy Glucose Sensor

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Abstract

Background: In this report, we detail our current work towards developing a surface-enhanced Raman spectroscopy (SERS) based sensor for in vivo glucose detection. Despite years of innovations in the development of blood glucose monitors, there remains a need for accurate continuous glucose sensors to provide care to rising numbers of diagnosed diabetes patients and mitigate secondary health complications associated with this metabolic disorder.

Methods: SERS is a highly specific and sensitive optical technique suitable for direct detection of glucose. The SERS effect is highly distance dependent, thus the glucose molecules need to be within a few nanometers or adsorbed to an SERS-active surface. In our sensor, this is achieved with a self-assembled monolayer (SAM) that facilitates reversible interactions between glucose molecules and the surface. The amount of glucose near the surface is proportional to its concentration in the surrounding environment.

Results: We determined that the SAM-functionalized surface is stable for at least 10 days and provides rapid, reversible partitioning. In vitro experiments in bovine plasma as well as in vivo experiments in rats demonstrated quantitative detection.

Conclusions: We show successful use of the SERS glucose sensor in rats, making it the first in vivo SERS sensor. Furthermore, we demonstrate free space transdermal detection of a SERS signal through the rat's skin as an initial step toward developing a transcutaneous sensor.

Introduction

TIGHT GLYCEMIC CONTROL remains the primary goal in successful diabetes management. This can be accomplished through a combination of regimented diet, exercise, insulin administration, and frequent blood glucose measurements. The latter is crucial in order to make informed decisions regarding the treatment plan (diet, medicine, etc.) and to avoid secondary health complications due to diabetes that are currently on the rise. Frequent monitoring of glucose levels can also provide important insight into the fundamental mechanisms of the disorder and help physicians establish trends in their patients' glucose fluctuations. This can mitigate long-term hyperglycemic conditions as well as avoid dangerous nocturnal hypoglycemia. Despite ~40 years of innovation in this field, the development of a continuous glucose monitor remains a worldwide goal. Recent advances in the fields of

photonics and computing afford the opportunity to explore many novel optical approaches to achieve a continuous glucose sensor. This paper will discuss the advances made toward the development of a continuous glucose sensor based on surface-enhanced Raman spectroscopy (SERS).

Glucose detection methods

The first blood glucose meter was introduced in 1969 and was known as the Ames Reflectance Meter. It was not designed for home use, and was primarily used in hospitals in the 1970s. The first devices that allowed patients to measure the blood glucose levels at home became available in the beginning of the 1980s, from companies such as Bayer HealthCare (Tarrytown, NY) and Roche Diagnostics (Indianapolis, IN). They consisted of an enzymatic test strip and a transducer-detector unit and required a drop of blood to be placed

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on the strip for the measurement to occur. These companies, as well as several others, continue to manufacture glucose monitoring devices today, and the method for blood glucose detection remains, in essence, the same. The sensors utilize enzymes, such as glucose oxidase, to produce a secondary product that can be electrochemically detected. Although the enzyme is inherently specific, the electrochemical measurement is nonspecific. It depends on the flux of glucose and other reaction substrates, and its signal can be mediated by a number of other electrochemically active species present in plasma or interstitial fluid. Over the years, these systems have become much more compact, require less blood volume, can manage recorded data, and communicate wirelessly with other devices.^{1–3} However, these sensors still suffer from insufficient frequency of measurements and—invariably—pain associated with drawing blood. Currently, there are several commercially available continuous glucose monitors, which sample interstitial fluid and measure glucose every few minutes. However, the inherent characteristics of electrochemical detection remain unchanged, and the use of these continuous glucose monitors is limited in routine diabetes care because of their cost and insufficient accuracy.⁴

Progress toward optical glucose detection began in the 1980s with laser polarimetry.^{5,6} Polarimetric glucose detection has been demonstrated in vitro and on an excised human eye.^{7–9} However, interference from other chiral molecules in the eye as well as corneal birefringence make in vivo detection difficult to implement.¹⁰ Another optical method for glucose detection is based on fluorescence.^{11–17} Fluorescent probes use exogenous chemical agents (fluorophores, enzymes, affinity-binding proteins) to measure glucose concentrations with great selectivity and sensitivity, although indirectly. It is challenging to provide biocompatible and stable encapsulation for fluorescent probes.¹⁶ Infrared (IR) absorption spectroscopy also has the potential for continuous glucose monitoring.^{18–25} The main challenge in IR spectroscopy is competing absorption by water and spectral overlap. Furthermore, the lack of a miniaturized broad-band light source at the moment makes it unfeasible to produce portable devices for home use. Normal Raman spectroscopy can also be used for glucose detection.^{26–31} Raman scattering by water is weak and does not interfere with the glucose spectrum. However, Raman spectroscopy suffers from inherently weak signals. Sufficient sensitivity can be achieved by SERS.

Raman and SERS

Raman spectroscopy is based on inelastic scattering of photons. When monochromatic light interacts with a molecule, the Raman effect causes energy shifts of the photons proportional to the vibrational energy of molecular bonds. In a Raman spectrum each band is characteristic of a specific intramolecular motion. Sharp linewidths over a broad spectral range and unique vibrational features in Raman spectroscopy provide very selective information, such that each molecule has its own unique Raman fingerprint. The specificity of Raman spectroscopy is demonstrated in Figure 1, with the Raman spectra of galactose (Fig. 1A) and glucose (Fig. 1B), C4 epimers. Both monosaccharides have the same chemical composition but differ structurally in the position

FIG. 1. Raman spectra of (A) galactose and (B) glucose, both crystalline. Despite extremely similar chemical structures, each molecule has a distinctly different Raman fingerprint. $\lambda_{\text{ex}} = 532 \text{ nm}$, $P = 20 \text{ mW}$, $t_{\text{ac}} = 2 \text{ min}$.

of one atom. Yet, they can be easily distinguished from each other by their Raman spectra. The main drawback of Raman spectroscopy is that it lacks the sensitivity required for rapid glucose detection at physiological concentrations. To achieve the requisite sensitivity, normal Raman spectroscopy would require long acquisition times and high powers not appropriate in most in vivo biomedical applications.

To overcome this limitation, VanDuyne and co-workers proposed an approach to measure glucose using SERS.³² The collective oscillation of free electrons on the metal surface excited by incident light causes selective absorption and scattering and is known as the localized surface plasmon resonance (LSPR). This results in the electromagnetic enhancement of Raman scattering with enhancement factor on the order of 10^6 – 10^8 .^{33,34} For single molecules, enhancement factors can be as high as 10^{14} .^{35–37} SERS is observed when molecules are within the decay length of the electromagnetic fields, even if the molecules are not chemisorbed.³⁴

The LSPR is highly dependent on the morphology of the metal nanostructure.^{38–41} Thus, careful control of the nanostructured metal surface structure is important in achieving reproducible enhancement. The spectral location of the LSPR significantly affects the intensity of the resulting SERS spectrum, which can be tuned by varying the height or width of the particles.⁴² In fact, SERS intensity is optimized when the laser excitation is slightly to the blue of the LSPR maximum.³⁷ As a result, both excitation and scattered photons are enhanced. Thus, the sensitivity is increased, and lower detection limits can be reached.

The SERS-active surfaces that we use for glucose sensing are known as film over nanospheres (FON). FON surfaces are fabricated by drop-coating an aqueous suspension of polystyrene nanospheres onto a supporting substrate, allowing the spheres to self-assemble into a close-packed array, and depositing a layer of metal, approximately 200 nm thick, to cover the spheres (Fig. 2A). SERS active surfaces were fabricated on chemically pure titanium substrates cut into 18-mm-diameter disks. Titanium and various titanium

FIG. 2. Components of the SERS glucose sensor. (A) Fabrication of FON surface. Silver is deposited onto the close-packed array of nanospheres. Atomic force micrograph of the FON surface. (B) Surface functionalization with SAM. The SAM comprised DT and MH partitions and departitions glucose.

alloys are biocompatible materials included in the Food and Drug Administration standards for implantable devices.⁴³

Surface modification with self-assembled monolayers (SAMs)

The glucose biosensor presented here uses SERS as the sensing modality.^{32,44–46} A silver FON (AgFON) surface provides maximum enhancement in the visible wavelength range,³⁴ although gold FON (AuFON) surfaces have also been evaluated as potential candidates for improving stability of the surfaces.⁴⁵ In the red and near-IR, where tissue absorption and scattering are diminished, gold and silver demonstrate comparable enhancement, making AuFON substrates viable candidates for future in vivo use of the sensor.

Bare metal surfaces have low affinity for glucose, and at physiological concentrations, there is insufficient glucose near the surface to produce a detectable SERS signal.^{32,47} To combat this limitation, the surface of the sensor is chemically

functionalized with a SAM to increase the affinity between the surface and glucose molecules (Fig. 2B). In initial studies, straight-chain alkanethiols were found to be the most effective in partitioning glucose,³² in particular, 1-decanethiol [$\text{HS}(\text{CH}_2)_9\text{CH}_3$]. As glucose partitions into the SAM, a signal from glucose can be observed.

The most recent work in optimizing the surface properties of the SERS glucose sensor resulted in the development of a novel two-component SAM (Fig. 2B). This combats the synthetic challenges associated with previously examined ethylene glycol-terminated alkanethiols.⁴⁴ The SAM is comprised of 1-decanethiol [$\text{HS}(\text{CH}_2)_9\text{CH}_3$] and 6-mercapto-1-hexanol [$\text{HS}(\text{CH}_2)_6\text{OH}$], which provide the appropriate hydrophilic/hydrophobic combination for glucose partitioning in aqueous solutions.⁴⁶ The decanethiol/mercaptohexanol (DT/MH) SAM is formed by incubating AgFON substrates first in 1 mM DT for 45 min and then transferring to 1 mM MH for at least 12 h.

Experimental Procedures

SERS instrumentation

A 785-nm wavelength laser was used in all experiments unless specified otherwise. This excitation wavelength was chosen to minimize autofluorescence of proteins.^{48,49} This is essential for experiments in the bovine plasma environment and in vivo detection. The substrates were either placed in a small-volume flow cell to control the external environment of the AgFON surfaces or implanted in a rat. Glucose solutions (at various concentrations) were injected into the flow cell containing the substrate at least 5 min prior to spectra acquisition. The sensor surface was not rinsed between exposures to different concentrations unless background spectra were part of the experimental design. Glucose levels in the animals were varied by intravenous glucose infusion. The schematic of the experimental setup is shown in Figure 3.

Animal protocol

Sprague-Dawley rats were anesthetized with isoflurane (1.5–3%) throughout the surgical procedure and the duration of the experiment. The animals were checked for pain reactions by toe-tug and blink tests. After the anesthetic had taken effect, the surgical areas were prepared by removal of hair and cleaning. The femoral vein and artery were cannu-

FIG. 3. Bench-top Raman spectroscopy system. The flow cell for in vitro work and the rat for in vivo work are interchangeable.

lated for drug/glucose injections and blood glucose measurements, respectively. Blood glucose measurements were made with Food and Drug Administration-certified home medical equipment (Ascensia® ELITE®, Bayer HealthCare). The animals were thermally stabilized by an electric heating pad throughout the course of the surgery and experiment. A metal frame containing a glass window was placed along the midline of the rat's back. A circular incision was made to allow the positioning of a DT/MH-functionalized AgFON sensor subcutaneously such that the substrate was in contact with the interstitial fluid and optically accessible through the window. Data were collected with the conventional Raman system described above (Fig. 3) with the rodent positioned in place of the flow cell. Following the experiment, the animals were sacrificed with an overdose of sodium pentobarbital (150 mg/kg), and death was assured by performing a bilateral thoracotomy.

Results

In vitro reversibility and stability of SERS sensor

An *in vivo* continuous sensor must be reversible and stable and also have a rapid response time. To demonstrate the reversibility of the sensor, the DT/MH-modified AgFON sensor was exposed to cycles of 0 and 100 mM buffered (pH ~7.4) aqueous glucose solutions (Fig. 4, inset). Each solution was injected into the flow cell and allowed to incubate for 2 min before acquiring spectra. There was no rinsing between steps in order to simulate real-time detection. Nitrate was added to the glucose solution as an internal standard (1053 cm^{-1} peak). The 1053 cm^{-1} band was used to normalize the spectra to minimize intensity fluctuations due to laser power variability. SER spectra were collected for each step ($\lambda_{\text{ex}} = 532 \text{ nm}$, $P = 10 \text{ mW}$, $t_{\text{ac}} = 20 \text{ min}$) (Fig. 4A–D). Figure 4E shows the normal Raman spectrum of a saturated aqueous glucose solution for comparison. In the normal Raman spec-

trum of a saturated aqueous glucose solution, the labeled peaks correspond to crystalline glucose peaks.⁵⁰ The difference spectra found by subtracting low concentration steps from high concentration steps (spectra B – A, D – C, Fig. 4F and G) represent partitioning of glucose in the DT/MH SAM. The spectral features match the peaks in the normal Raman spectrum of glucose (Fig. 4E). Literature has shown that SERS bands can shift up to 25 cm^{-1} when compared to normal Raman bands of the same analyte.⁵¹ The sharp peak seen in all of the difference spectra at 1053 cm^{-1} represents imperfect subtraction of the nitrate internal standard. The absence of glucose spectral features in the difference spectra found by subtracting steps with the same concentration from each other (spectra C – A, D – B, Fig. 4H and I) represents complete departitioning of glucose. The DT/MH mixed SAM presents a completely reversible sensing surface for optimal partitioning and departitioning of glucose.

The stability of the DT/MH-functionalized AgFON surface was also demonstrated for 10 days in bovine plasma.⁴⁶ SERS spectra were captured over a period of 10 days on three different DT/MH-functionalized surfaces. The average intensity of the 1119 cm^{-1} band was used as a metric of stability since it indicates that the SAM surface coverage as well as the SERS activity of the surface remain intact. The change in intensity of the 1119 cm^{-1} peak from the first day to the last day was 2.08%, indicating that it did not vary significantly during the 10-day period. The 2% decrease in the intensity can be attributed to the rearrangement of the SAM during incubation in bovine plasma.⁵² The temporal stability of the 1119 cm^{-1} peak intensity indicates that the DT/MH SAM was intact and well ordered, making this SAM-functionalized surface a viable candidate for an implantable sensor.

Another important characteristic of a sensor is fast response. It was determined that the $1/e$ time constants for partitioning and departitioning of the SERS-based glucose

FIG. 4. Glucose pulsing sequence (inset). (A–D) SER spectra of glucose solutions cycled between 0 and 100 mM. (E) Reference spectra of crystalline glucose. (F–I) Difference spectra showing partitioning and departitioning of glucose. For all trials, $\lambda_{\text{ex}} = 532 \text{ nm}$, $P = 10 \text{ mW}$, $t_{\text{ac}} = 20 \text{ min}$. Reprinted with permission from Lyandres et al.⁴⁶ Copyright ©2005 American Chemical Society.

sensor are less than 30 s.⁴⁶ The time constant was determined by introducing a step change in concentration from 0 to 50 mM and back down to 0. Spectra were acquired every 15 s, and the amplitude of the 1462 cm^{-1} glucose band was determined at each of those time points. After an exponential curve was fitted to the points, the time constants were demonstrated to be less than 30 s for partitioning and departitioning. The SERS glucose sensor characteristics so far make it a viable candidate for in vivo continuous detection. It is reversible, stable, and has a rapid time response to fluctuations in concentration levels.

Quantitative glucose detection in vitro

In order for a glucose sensor to be viable, it should be able to detect glucose in the clinically relevant range 10–450 mg/dL (0.56–25 mM), under physiological pH, and in an environment simulating in vivo conditions. The calibration data are presented in the Clarke error grid, the most common standard for evaluating the performance of glucose sensors for the clinically relevant concentration range (0–450 mg/dL).⁵³ Data points that fall in zones A and B are acceptable values. Values outside zones A and B result in erroneous diagnosis and/or potential failure to detect blood glucose levels outside of the target range. Bovine plasma was

used to simulate the in vivo environment of an implantable glucose sensor. Bovine plasma was spiked with glucose resulting concentrations ranging from 10 to 450 mg/dL. DT/MH-functionalized AgFON substrates were placed in the flow cell and exposed to the glucose-spiked bovine plasma. SER spectra were collected at each concentration using multiple samples and multiple spots in random order to construct a robust calibration model ($\lambda_{\text{ex}} = 785 \text{ nm}$, $P = 10\text{--}30 \text{ mW}$, $t_{\text{ac}} = 2 \text{ min}$). Data analysis was performed using standard chemometric methods. The calibration model was constructed using the partial least-squares (PLS) analysis and leave-one-out (LOO) cross validation algorithm resulting seven latent variables and presented on a Clarke error grid (Fig. 5). To construct the calibration, 92 randomly chosen data points were used, resulting in a root mean squared error of calibration (RMSEC) of 34.3 mg/dL (1.90 mM).

In addition to having a low RMSEC, it is important to use an independent validation set to test the calibration model.⁵⁴ For the validation, 46 data points were used resulting a root mean squared error of prediction (RMSEP) of 83.16 mg/dL (4.62 mM). On the Clarke error grid, 98% for calibration and 85% for validation fall in the zone A and B range. Error can be attributed to variation in SERS enhancement at different spots and different substrates.⁵⁵ The RMSEP can be improved by increasing the number of data points in the calibration set. The results show that the DT/MH-modified AgFON glucose sensor is capable of making accurate glucose measurements in the presence of many interfering analytes.

Quantitative glucose detection in vivo

DT/MH-modified AgFONs were implanted in rats as described above. Glucose levels were varied in the rat through intermittent intravenous infusion (1 g/mL in phosphate-buffered saline) over the course of the experiment. A droplet of blood was drawn from the rat, the glucose level was measured with the Ascensia Elite glucose meter, and corresponding SERS measurements were taken. To keep the osmotic pressure of the rat at normal physiological levels, a volume of bovine serum albumin equal to the blood removed was injected following each blood glucose measurement. The SERS spectra were acquired through the implanted optical window.

Four separate in vivo experiments are presented on Clarke error grids in Figure 6. All measurements were taken from a single spot on the implanted sensor and analyzed using the techniques described above. The results are summarized in Table 1. For each experiment, all points of the calibration and validation fall in the zone A and B range, showing that our SERS sensor can make accurate glucose measurements in vivo.

Real-time glucose detection in vivo

In addition to accurate quantitative predictions, it is important to verify that the SERS sensor is able to track changes in glucose levels over time. We compared the glucose concentrations predicted by the SERS sensor and those predicted by Ascensia ELITE, a commercial electrochemical sensor. Figure 7 shows the variation in glucose as a function of time measured over the course of two experiments. Glucose concentration was varied artificially by infusing glucose solution or insulin into the animal. The blood glucose concentration was measured simulta-

FIG. 5. PLS calibration (◆) and validation (●) plotted on a Clarke error grid. The “normal” and “pre-diabetes” blood glucose levels are defined according to the oral glucose tolerance test. The calibration model was constructed using 92 data points. The validation was performed using 46 data points taken over a range of glucose concentrations (10–450 mg/dL) in bovine plasma. RMSEC = 34.3 mg/dL (1.9 mM), RMSEP = 83.16 mg/dL (4.62 mM). $\lambda_{\text{ex}} = 785 \text{ nm}$, $P_{\text{laser}} = 10\text{--}30 \text{ mW}$, and $t_{\text{ac}} = 2 \text{ min}$. Reprinted with permission from Lyandres et al.⁴⁶ Copyright ©2005 American Chemical Society.

FIG. 6. PLS calibration (◆) and validation (●) plotted on a Clarke error grid for rats 1–4. For Rat 1, the calibration model was constructed using 20 data points, and the validation set utilized seven data points. For Rat 2, the calibration was constructed using 30 data points correlated, and the validation set utilized 10 data points. For Rat 3, the calibration was constructed using 25 data points, and the validation set utilized 10 data points. For Rat 4, the calibration was constructed using 27 data points, and the validation set utilized 10 data points. All data points were acquired in vivo with $\lambda_{\text{ex}} = 785 \text{ nm}$, $P = 50 \text{ mW}$, $t_{\text{ac}} = 2 \text{ min}$.

neously with the Ascensia ELITE blood glucose meter, and the SERS glucose sensor ($\lambda_{\text{ex}} = 785 \text{ nm}$, $P = 50 \text{ mW}$, $t_{\text{ac}} = 2 \text{ min}$). Figure 7A shows the case where glucose concentration remained relatively constant despite glucose infusion in the animal. Figure 7B shows fluctuations in glucose levels as a result of glucose infusion beginning at approximately 77 min and administering of insulin at approximately 208 min.

Both the standard glucometer and the SERS-based measurements effectively tracked the fluctuations in glucose concentration. Though the concentration values do not agree perfectly, the results indicate that the same trends can be observed with both sensors. Careful consideration must be given to the calibration method and its result on the accuracy of the sensor. The Ascensia ELITE glucometer (also used for calibrating the SERS sensor) measures blood glucose con-

TABLE 1. IN VIVO QUANTITATIVE DETECTION USING PLS CALIBRATION

Rat number	Points used for		RMSEC	RMSEP
	Calibration	Validation		
1	20	7	8.9 mg/dL (0.5 mM)	23.6 mg/dL (1.3 mM)
2	30	10	10.3 mg/dL (0.6 mM)	72 mg/dL (4 mM)
3	25	10	10.2 mg/dL (0.6 mM)	64.5 mg/dL (3.6 mM)
4	27	10	11.3 mg/dL (0.6 mM)	80.9 mg/dL (4.5 mM)

Each calibration was based on data acquired from one animal. The RMSEC and RMSEP values are comparable to values previously reported for the SERS and other optical glucose techniques.

FIG. 7. Glucose concentration versus time measurements comparing a commercial electrochemical glucose meter (blood, $\blacktriangle$), and the SERS sensor (interstitial fluid, $\blacksquare$). Glucose levels are qualitatively similar for both sensors. In Trial 1, glucose infusion (G) was started at min 44 (although glucose levels remained low). In Trial 2, glucose infusion (G) was started at min 77, and insulin (I) was administered at min 208 as confirmed by the spike and a drop in glucose concentrations, respectively.

centration, while the SERS sensor is detecting glucose in the interstitial fluid. There is a delay associated with diffusion of glucose from vessels into tissues. The difference between blood and interstitial glucose levels is large during rapid changes of glucose in the body, for example, following a meal or after intense exercise. In the experiments, a glucose solution was infused into the rats' vasculature, resembling food intake. Therefore, it is not surprising that the concentrations predicted by the Ascensia glucometer and the SERS glucose sensor do not agree quantitatively. However, these data successfully demonstrate that the SERS glucose sensor remains viable in vivo and can detect fluctuations in glucose levels as a function of time.

Toward transdermal in vivo detection of glucose

Since SERS measurements require that the analyte interacts with the surface, the resulting sensor would be implanted subcutaneously for measurements, i.e., minimally invasive. While we are currently using 18-mm-diameter supporting substrates, the actual surface probed is equal to the laser spot size and therefore can be reduced to less than 100 μm in diameter. One method of introducing the sensor into the body is by inserting a catheter with a fiber optic probe, which would have a SERS-active substrate attached to it. However, we hope that the implanted sensor can be addressed transdermally, without the need for additional surgical procedures. Herein, we successfully demonstrate that a SERS signal from an AgFON sensor can, indeed, be detected through the skin of a rat.

Shaved skin was obtained from the belly of a male Sprague-Dawley rat. Fat and fascia were removed from the skin sample. An AgFON functionalized with a monolayer of benzenethiol (BZT) was characterized in the conventional Raman setup described above. BZT was chosen because of its large Raman cross-section. Spectra acquired prior to placement of skin is shown in Figure 8A ($\lambda_{\text{ex}} = 785 \text{ nm}$, $P = 50 \text{ mW}$, $t_{\text{ac}} = 2 \text{ min}$). The skin was then placed in contact with the AgFON such that both the excitation beam and Raman scattered photons passed through the skin. Spectra acquired with rat skin covering the BZT functionalized AgFON is shown in Figure 8B. The labeled BZT peaks can be clearly seen in both spectra. The intensity of the Raman signal is, however, reduced by a factor of 130. Since the Raman cross-section of glucose is only approximately five times smaller than that of BZT,⁵⁶ these data suggest that transdermal detection of glucose may also be possible. Furthermore, there is evidence that use of optical clearing agents such as glyce-

rol have the potential to improve photon transmission through dermal tissues.⁵⁷ In addition, fiber optic probes developed by Matousek and co-workers for spatially offset Raman spectroscopy provide chemically specific information on deep layers of human tissue and appear extremely promising.^{58,59}

Conclusions

We have successfully demonstrated the feasibility of an SERS biosensor for continuous in vivo detection of glucose. Until recently, we have concentrated on developing and understanding the characteristics of the sensor in the laboratory environment, showing that the SERS-active surface provides a stable, reversible, and quantitative platform for glucose detection. However, the most compelling evidence that the SERS sensor could be developed for future human use is the latest demonstration that the sensor functions successfully in vivo. In the future, we will focus on evaluating the biocompatibility of the sensor and its performance in

FIG. 8. Transdermal SERS measurements. (A) Spectrum of a BZT-functionalized AgFON. (B) Spectrum of a BZT-functionalized AgFON taken through rat skin. A 130-fold decrease in intensity is observed. $\lambda_{\text{ex}} = 785 \text{ nm}$, $P_{\text{laser}} = 50 \text{ mW}$, and $t_{\text{ac}} = 2 \text{ min}$. a.u., arbitrary units.

vivo and the development of a miniaturized transdermal detection scheme, as well as continuing our work of examining the fundamental properties of the sensor, in vitro.

Acknowledgments

Funding for this work was provided by the National Institutes of Health (grant DK066990-02), the U.S. Army Medical Research and Materiel Command's Military Operational Medical Research Program/Julia Weaver Fund (grant W81XWH-04-1-0630), the National Science Foundation (grant CHE0414554), and the Air Force Office of Scientific Research MURI program (grant F49620-02-1-0381).

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