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Electrochemical Aptamer-Based Sensors for Improved Therapeutic Drug Monitoring and High-Precision, Feedback-Controlled Drug Delivery

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Abstract

The electrochemical aptamer-based (E-AB) sensing platform appears to be a convenient (rapid, single-step, and calibration-free) and modular approach to measure concentrations of specific molecules (irrespective of their chemical reactivity) directly in blood and even in situ in the living

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ASSOCIATED CONTENT

Supporting Information

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Materials, chemicals, and experimental methods for the development of the vancomycin-binding E-AB sensors, including the selection of the aptamer, electrochemical characterization of the sensor with the calibration-free parameters, sensor's response time, and PID control of lower vancomycin doses (PDF)

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body. Given these attributes, the platform may thus provide significant opportunities to render therapeutic drug monitoring (the clinical practice in which dosing is adjusted in response to plasma drug measurements) as frequent and convenient as the measurement of blood sugar has become for diabetics. The ability to measure arbitrary molecules in the body in real time could even enable closed-loop feedback control over plasma drug levels in a manner analogous to the recently commercialized controlled blood sugar systems. As initial exploration of this, we describe here the selection of an aptamer against vancomycin, a narrow therapeutic window antibiotic for which therapeutic monitoring is a critical part of the standard of care, and its adaptation into an electrochemical aptamer-based (E-AB) sensor. Using this sensor, we then demonstrate: (i) rapid (seconds) and convenient (single-step and calibration-free) measurement of plasma vancomycin in finger-prick-scale samples of whole blood, (ii) high-precision measurement of subject-specific vancomycin pharmacokinetics (in a rat animal model), and (iii) high-precision, closed-loop feedback control over plasma levels of the drug (in a rat animal model). The ability to not only track (with continuous-glucose-monitor-like measurement frequency and convenience) but also actively control plasma drug levels provides an unprecedented route toward improving therapeutic drug monitoring and, more generally, the personalized, high-precision delivery of pharmacological interventions.

Graphical Abstract:

Keywords

vancomycin; therapeutic drug monitoring; controlled drug delivery; square-wave voltammetry; DNA aptamer; electrochemical DNA biosensor

INTRODUCTION

The therapeutic windows (range of plasma concentrations over which a molecule is therapeutically effective without causing significant adverse effects) of most drugs are wide relative to typical metabolic variability, and thus indirect predictors of patient metabolism, such as age, body mass, or genotype, are sufficient to ensure effective dosing.^{1–3} The therapeutic windows of many drugs, however, are narrow relative to interpatient metabolic variability, and the accuracy of indirect pharmacokinetic predictors is insufficient to ensure safe dose determination and drug delivery.^{1,4,5} Under these circumstances, therapeutic drug monitoring (TDM), the clinical practice in which plasma drug concentrations are measured to adjust dosing, remains the standard of care.^{6–8} This is particularly true for the most

grievously ill patients as this is the population most likely to exhibit altered metabolism and the population for which the margin for therapeutic error is narrowest.^{9–11}

The need for and problems associated with TDM are compellingly illustrated by the difficulty in properly delivering vancomycin, a “key access antibiotic”. Because plasma levels below the drug’s therapeutic window can lead to dangerously poor efficacy (e.g., death due to sepsis) and those above the window are associated with permanent hearing loss and nephrotoxicity, TDM is a recommended element of the standard of care in vancomycin treatment.^{12–15} Current methods of monitoring plasma vancomycin levels, however, rely on venous blood draws that are analyzed using immunoassays^{16,17} or HPLC-MS^{16–19} and are thus slow, cumbersome processes requiring fully equipped laboratories and returning an answer only hours to days after sample collection (Figure 1A). As a result, TDM of vancomycin is performed more rarely than is optimal^{2,12,13,15} and, even when performed, provides only a low resolution (a few data points) “snapshot” that may not accurately measure a patient’s metabolism^{20–23} (Figure 1A). More generally, the slow, cumbersome nature of current drug measurement approaches remains a major hurdle hindering the wider adoption of TDM.^{20,24,25} Against this background, our goal is to develop a platform that renders plasma drug level measurements as easy and convenient as current blood sugar measurements under the argument that at-home finger-prick measurements of plasma drug levels (Figure 1B) or, ultimately, continuous, real-time measurements performed via a wearable device (Figure 1C) would vastly improve our ability to accurately dose and properly deliver this and many other pharmacological therapies (Figure 1D).

In response to the need for improved methods of performing TDM, we have developed electrochemical aptamer-based (E-AB) sensors, a reagentless, single-step sensing platform for the rapid, and even real-time, measurement of plasma drug levels in whole blood and even directly in the body. E-AB sensors are composed of an electrode-bound redox-reporter-modified aptamer sequence that undergoes a binding-induced change in electron transfer kinetics easily monitored using, for example, square-wave voltammetry.^{28–30} In this paper, we explore the potential of the E-AB platform as a means of performing TDM by selecting the first reported aptamer against vancomycin and adapting it into an E-AB sensor. We then applied this new sensor to (i) the rapid, calibration-free quantification of vancomycin *ex vivo* in unprocessed, finger-prick-scale volumes of whole blood; (ii) achieving seconds-resolved, high-precision vancomycin pharmacokinetic measurements; and (iii) performing high-precision feedback control over plasma levels of this historically difficult-to-dose drug.

RESULTS AND DISCUSSION

E-AB sensor development requires the availability of an aptamer that achieves clinically relevant affinity and specificity. To identify such an aptamer, we performed a solution-phase selection based on a stem-loop closing scheme where the details of which can be found in the Supporting Information and in prior works.^{31,32} This selection starts with a random DNA library of $\sim 6 \times 10^{14}$ unique sequences in which each member is composed of a 45-base random element flanked by two partially complementary primers (Figure 2A). As previously reported,^{33,34} the library members are attached to an agarose–streptavidin solid support via hybridization to a biotinylated anchor strand complementary to one of the two primers,

which also prevents the primers from hybridizing with one another to form a double-stranded stem.^{31,32,35} Sequences that undergo a binding-induced conformational change leading to detachment (presumably due to stabilization of the stem) are then collected, amplified, and regenerated. Repeating this cycle 14 times, we produced an aptamer against vancomycin with a dissociation constant (K_D) of $\sim 0.1 \mu\text{M}$ when measured free in a bulk solution using a previously described³² optical assay (Figure S1).

To support E-AB sensing, an aptamer must undergo a binding-induced conformational change that, in turn, produces a significant change in electron transfer kinetics (Figure 2B). The full-length (“parent”) aptamer does not exhibit this property (Figure S3A), presumably due to the stability of the folded structure. Circular dichroism studies suggest, however, that the removal of four base pairs from the stem produces the necessary binding-induced conformational change (Figure S3B). Consistent with this, when adapted into the E-AB platform, the truncated aptamer supports good E-AB signaling, exhibiting a relative signal change upon the addition of a saturating target of $\sim 120\%$ and a K_D of $45 \mu\text{M}$ when challenged in undiluted flowing whole blood (Figure 2C). These attributes support vancomycin measurements across the entire $6\text{--}35 \mu\text{M}$ clinical range of the antibiotic.³⁶ The sensor is also rapid: when challenged with $10 \mu\text{M}$ vancomycin, it completely equilibrates in the time (9 s) it takes to acquire a pair of square-wave voltammograms (Figure S4).

By analogy to the impact that the home glucose monitor has had on diabetes care, the E-AB platform could significantly improve on current TDM methods by moving it from laboratory-based tests to at-home “self-testing” (Figure 1B). To explore this possibility, we fabricated vancomycin-detecting sensors using wire electrodes small enough to immerse in sub- $100 \mu\text{L}$ volumes (Figure 3A) and then deployed them in a calibration-free manner.²⁹ Briefly, this relies on the strong square-wave frequency dependence of E-AB signaling (Figure 2C) to produce a “nonresponsive” current that is independent of the target concentration used to correct for sensor-to-sensor variation.^{28–30} Using this approach to measure vancomycin spiked into $100 \mu\text{L}$ samples of whole bovine blood returns concentration estimates within $\pm 20\%$ of the known concentration of the drug over the entire clinically relevant range (Figure 3B), suggesting that E-AB sensors support convenient, calibration-free, and finger-prick-style self-testing closely analogous to the home-glucose-meter testing employed by many diabetics.

Although self-testing of plasma vancomycin levels using finger-prick samples could improve the convenience of TDM, such measurements still only provide poorly time-resolved “snapshots” of metabolism. In contrast, the ability of indwelling E-AB sensors to achieve subsecond-resolved measurements directly in the living body^{37–39} would provide an unprecedented route toward not only measuring plasma drug levels but also measuring patient-specific pharmacokinetics. To explore this, we placed E-AB sensors in the veins of live Sprague–Dawley rats via a 22 gauge catheter (Figure 4A). To remove the drift seen in *in vivo* deployments, we used a previously described drift correction approach: kinetic differential measurements (KDM).^{38–40} This takes the difference between the sensor’s response measured at two square-wave frequencies—one in which the sensor is “signal-on” and the other at which the sensor is “signal-off”—but that drift in concert.⁴⁰ Using KDM-corrected sensors can measure 9 s-resolved plasma vancomycin levels in real time after, for

example, the intravenous injection of human-equivalent, 30 mg kg⁻¹ doses (Figure 4B). The resultant pharmacokinetic curves obtained from this experiment produces peak concentrations and plasma half-lives consistent with values reported in the literature.⁴¹ The typical ~15 min time resolution of these prior (research, not clinical) measurements, however, is poorly matched to the metabolic timescale of the drug, and thus the precision with which they define its distribution (α) and elimination (β) time constants is limited.²⁶ In contrast, high-frequency E-AB measurements return parameter estimates with precision (defined as 95% confidence intervals) of better than 20%, which is more than sufficient to identify statistically significant pharmacokinetic differences between individual animals (Figure 4B).

The goal of TDM is to increase the time during which plasma drug levels remain within a drug's therapeutic window.²⁵ The ultimate realization of this would be to measure these levels in real time and then use this information to optimize dosing on a timescale faster than the metabolic timescale of the drug. The seconds-resolved, real-time measurements provided by indwelling E-AB sensors support just such feedback-controlled delivery.³⁸ To illustrate this, we placed an indwelling vancomycin sensor into the right jugular vein of a rat and a drug-delivery catheter into the left jugular vein that was attached to a syringe pump (Figure 5A). We then employed the sensor's output to drive a proportional–integral–derivative (PID) controller that, in turn, adjusted the flow rate of the syringe pump every 9 s. Using this system, we rapidly achieve (~30 min) and accurately maintain ($\pm 2 \mu\text{M}$) plasma vancomycin concentrations over the course of hours (Figure 5B and Figure S4) even in the face of several-fold variations in the rate with which the drug is eliminated from the body during this same period (Figure 5C). Note that the limited duration of these experiments is due to animal welfare concerns (which preclude keeping rats under anesthesia for more than 6 h) and not by loss of sensor performance.

Here, we have used a rat animal model to explore the application of E-AB sensors to the problem of performing TDM and, as the ultimate goal in TDM, feedback-controlled, high-precision drug delivery. To do so, we have selected a vancomycin-binding aptamer via a solution-phase selection scheme followed by its reengineering and adaptation into an E-AB sensor. Using this sensor, we then performed (i) the rapid (seconds), convenient (single-step, calibration-free), and accurate ($\pm 20\%$) determination of vancomycin levels in finger-prick-scale volumes of unprocessed whole blood; (ii) the high-precision ($\pm 20\%$) measurement of subject-specific vancomycin pharmacokinetics; and (iii) the high-precision ($\pm 20\%$) feedback control of plasma vancomycin levels in the face of significant, hour-to-hour changes in drug metabolism.

TDM was introduced into clinical practice to counter the relatively narrow therapeutic windows of some drugs and, in doing so, to improve the safety and efficacy of treatments. TDM has been shown, for example, to significantly improve patient outcomes upon treatment by vancomycin and the aminoglycoside antibiotics.^{25,42} TDM can likewise improve patient compliance (less of a critical concern for vancomycin considering that it is administered/monitored in a hospital setting but is a serious health issue for many other drugs^{43,44}) and the cost-effectiveness of treatments.²⁵ Unfortunately, however, studies have repeatedly shown that clinicians often fail to achieve these goals,¹² suggesting that

improvements in our ability to perform TDM are a critical component of the move toward high-precision, personalized medicine.^{14,20} Specifically, we believe that the development of a platform technology for convenient measurements of plasma drug levels would represent a paradigm shift in how TDM is performed and could significantly improve outcomes, reduce complications, and reduce health costs. Next steps are currently being taken in order to translate E-AB sensors for longer term in vivo measurements so that they can be more easily applied in clinical settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Current approaches to therapeutic drug monitoring (TDM) rely on venous blood draws and subsequent analysis in centralized laboratories, which are slow and cumbersome and provide only a few points for monitoring drug metabolism. Specifically, vancomycin's standard of care requires a "peaks and troughs" measurement (i.e., the highest and lowest concentration of the drug) in which often complex, multiphase pharmacokinetics are fitted.^{26,27} Here, in contrast, we explore the ability of electrochemical aptamer-based (E-AB) sensors to improve on the convenience and accuracy of TDM. (B) For example, the ability of E-AB sensors to rapidly measure plasma drug levels using finger-prick volumes of unprocessed whole blood would enable self-testing and thus more frequently informed dosing. (C) Indwelling E-AB sensors supporting the high-frequency measurement of plasma drug levels could be used to determine patient-specific pharmacokinetics with unprecedented precision to (D) ultimately be used to perform feedback-controlled delivery in which dosing is optimized every minute in response to metabolic fluctuations.

Figure 2.

We demonstrate here the fabrication of a new, vancomycin-detecting E-AB sensor. (A) To achieve this, we first performed a solution-phase aptamer selection scheme that utilizes a key stem-loop closing step. Briefly, this starts with a single-stranded, surface-attached sequence partially complementary to a fixed region in a library pool of $\sim 10^{14}$ distinct sequences flanked with two amplification regions and a random region of 45 nucleotides (in gray). Sequences that respond to the presence of target by detaching from their complement (presumably due to an intramolecular binding-induced conformational change) are then recovered, amplified, reconverted into a single-stranded library, and subjected to more rounds of selection. To measure the affinity of the resulting aptamer, we modified its sequence with a fluorophore and hybridized it using the partially complementary quencher-modified sequence (Figure S1). Titrating this with vancomycin further releases the complement sequence, thus increasing the measured fluorescence producing a dissociation constant (K_D) of $\sim 0.1 \mu\text{M}$. (B) We adapted the aptamer to the E-AB platform, which relies on a binding-induced change in the electron transfer kinetics of a redox reporter-modified (i.e., represented as “R”, which stands for methylene blue), surface-attached aptamer to produce a change in electron transfer rate that can be monitored using square-wave voltammetry. (C) Due to the dependence of electron transfer kinetics on the presence of target, the relative response of the vancomycin sensor is a strong function of square-wave

frequency, producing “signal-off,” “signal-on,” or “nonresponsive” behaviors^{28–30} that cover the clinically relevant range concentrations³⁶ in undiluted, flowing whole blood. (D) Using the output at the nonresponsive frequency and the constants α (the ratio of the current at the nonresponsive frequency and at the responsive frequency at zero target concentration), K_D (the dissociation constant of the aptamer), and γ (signal gain) for vancomycin-detecting E-AB sensors (as a class), we can determine the concentration of vancomycin without the need for calibrating each individual device (see Table S1 for more details).²⁹ Doing so returns measured vancomycin concentrations within 20% of the applied concentration. The “error bars” shown here and those reported or illustrated elsewhere in this paper reflect 95% confidence intervals estimated from replicate measurements conducted on five independently fabricated sensors.

Figure 3.

Using $\sim 200\ \mu\text{m}$ -diameter, 3 mm-long (active sensing area) E-AB sensors, we have performed the calibration-free measurement of vancomycin in finger-prick-scale volumes ($100\ \mu\text{L}$) of whole blood. (A) To achieve this, we employed sensors fabricated using $75\ \mu\text{m}$ -diameter gold, silver, and platinum wires that can easily be immersed in small volumes. (B) Using the nonresponsive²⁹ square-wave frequency (Figure 2B), we achieve clinically relevant ($\pm 20\%$) precision in the measurement of vancomycin over its entire clinical concentration range via a rapid (seconds) and single step. The asymmetry of the error bars is due to the use of a logarithmic y axis.

Figure 4.

Vancomycin E-AB sensor supports the high-precision measurement of patient-specific pharmacokinetics. (A) We show this via the insertion of a fine-wire sensor into the right jugular vein (via a 22G catheter) of a rat. We then insert an infusion line in the left jugular vein through which we administer vancomycin intravenously. (B) Intravenous dose of 30 mg kg^{-1} produces biphasic kinetics with elimination (β) time constants resolved to a precision of better than 20% (which represents the error at 95% confidence interval of the fitted trace).

Figure 5.

E-AB measurements support feedback-controlled drug delivery, supporting in turn high-precision control over plasma drug levels. (A) For this, we use seconds-resolved E-AB measurements as the input to a proportional–integral–derivative (PID) controller that computes the dosing rate required to best reach the desired plasma concentration administered via a PID-controlled drug-delivery infuser. (B) Using this approach, we can maintain a plasma vancomycin concentration within 2 μM of, as shown here, a 10 μM set point for more than 5 h, a duration that is limited by animal welfare concerns rather than by sensor performance. (C) We maintain this concentration in the face of ~ 3 -fold variation in the drug's elimination rate (calculated using the instantaneous infusion rate estimated by the PID); the black trace represents a 27 s (i.e., 3-point) rolling average.