

Editor's Summary

Tracking Drugs in Real Time

You have the drug, it's time to give to the patient. Now, what is the ideal dose? Many drugs have unwanted side effects when given at large doses; conversely, they are not efficacious at too low of a dose. Continuously monitoring a drug as it circulates throughout the body would give doctors a better grip on personalized medicine, by allowing them to then tailor the therapeutic dose and schedule for each patient. To this end, Ferguson *et al.* developed a biosensor that reports the concentration of a drug in real time in live animals and in patient samples.

The microfluidic sensing device, which the authors named MEDIC (microfluidic electrochemical detector for in vivo continuous monitoring), consisted of an electrochemically modified aptamer—a oligonucleotide that is highly specific for a target drug—attached to a gold electrode, as well as a filter to prevent blood cells from clogging up the device. The electrodes reported the change in charge as the drug bound to the aptamer. Ferguson *et al.* used two different aptamers: one specific for doxorubicin (DOX; a cancer drug) and one for kanamycin (an antibiotic). The authors first demonstrated that MEDIC could detect submicromolar concentrations of DOX in human whole blood. The MEDIC was then hooked up to live rats to continuously draw blood for monitoring. Injecting the animals with a drug-free solution yielded no change in device signal. However, injecting therapeutically relevant doses of DOX or kanamycin—depending on the device configuration—quickly produced a signal that corresponded to the in vivo drug concentration.

Such continuous monitoring of drugs could afford clinicians the opportunity to tailor therapeutic regimens to individual patients, thus preventing toxic side effects or dialing up the drug effect. Translating this technology to people may require tweaking the sensor for longer operation times (days to weeks, versus the hours described here), as well as safety testing. Once deemed useful and safe, the device could replace periodic and disruptive blood draws at the patient's bedside, much like continuous glucose monitors in widespread use today for diabetes.

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Real-Time, Aptamer-Based Tracking of Circulating Therapeutic Agents in Living Animals

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A sensor capable of continuously measuring specific molecules in the bloodstream *in vivo* would give clinicians a valuable window into patients' health and their response to therapeutics. Such technology would enable truly personalized medicine, wherein therapeutic agents could be tailored with optimal doses for each patient to maximize efficacy and minimize side effects. Unfortunately, continuous, real-time measurement is currently only possible for a handful of targets, such as glucose, lactose, and oxygen, and the few existing platforms for continuous measurement are not generalizable for the monitoring of other analytes, such as small-molecule therapeutics. In response, we have developed a real-time biosensor capable of continuously tracking a wide range of circulating drugs in living subjects. Our microfluidic electrochemical detector for *in vivo* continuous monitoring (MEDIC) requires no exogenous reagents, operates at room temperature, and can be reconfigured to measure different target molecules by exchanging probes in a modular manner. To demonstrate the system's versatility, we measured therapeutic *in vivo* concentrations of doxorubicin (a chemotherapeutic) and kanamycin (an antibiotic) in live rats and in human whole blood for several hours with high sensitivity and specificity at subminute temporal resolution. We show that MEDIC can also obtain pharmacokinetic parameters for individual animals in real time. Accordingly, just as continuous glucose monitoring technology is currently revolutionizing diabetes care, we believe that MEDIC could be a powerful enabler for personalized medicine by ensuring delivery of optimal drug doses for individual patients based on direct detection of physiological parameters.

INTRODUCTION

The paradigm of personalized medicine holds the promise to revolutionize healthcare by delivering “the right drug, at the right dose, and at the right time” (1). Toward the realization of this potential, the past decade has witnessed significant advancements in the development of biosensors capable of sensitive and specific detection of small-molecule, nucleic acid, and protein targets (2, 3). A limitation of most of these sensors, however, is that they generally only perform single time-point measurements. This prevents their use for continuous, real-time monitoring of molecular analytes, an ability that could be of considerable medical value. Indeed, continuous, real-time molecular monitoring is available for only a handful of analytes that produce readily measureable signals from analyte-specific reactions (for example, detection of glucose through glucose oxidase activity) (4, 5). A universal architecture that can continuously measure *in vivo* concentrations of a wide range of circulating biomolecules would enable many potentially transformative applications in medicine; for example, continuous monitoring of cardiac markers (for example, troponin) could predict an oncoming heart attack (6), measurements of chemokines could provide early warning for infection or autoimmune flare-ups (7),

and real-time tracking of chemotherapeutic agents could facilitate administration of a therapeutic dose that is continuously optimized for maximal efficacy and minimal side effects for a specific cancer patient (8).

The design of such a sensor poses extraordinary technological challenges (9–11). First, the sensor must operate continuously, without sample preparation, batch processing, or addition of exogenous reagents. Second, it must achieve sufficient sensitivity, selectivity, and dynamic range and demonstrate the ability to resolve changes in analyte concentrations at physiological time scales. Finally, it must be resistant to fouling even after prolonged exposure to whole blood and other complex samples, and remain stable with high signal-to-noise ratios (SNRs) while operating in this environment for extended periods of time. To date, no platform has satisfactorily addressed these challenges, and there remains an urgent need for a generalizable approach to continuous *in vivo* detection of clinically relevant target molecules in real time.

In response, we have developed the microfluidic electrochemical detector for *in vivo* continuous monitoring (MEDIC)—a biosensor platform that can be readily reconfigured to continuously measure a diverse array of biomolecules in real time. As proof of concept, we have used MEDIC to measure *in vivo* concentrations of doxorubicin (DOX), a widely used chemotherapeutic, in human whole blood and in live rats (Fig. 1A). We chose DOX because it exhibits substantial, clinically meaningful changes in pharmacokinetics across populations, and even over the course of treatment for a single individual (12). By simply exchanging the probes in our MEDIC chip, we were also able to achieve real-time *in vivo* measurement of kanamycin, an antibiotic, demonstrating the inherent modularity of this platform.

The MEDIC system overcomes the long-standing limitations of real-time *in vivo* detection. By supporting measurement of a range of drugs and biomarkers, we expect MEDIC to elucidate individual and

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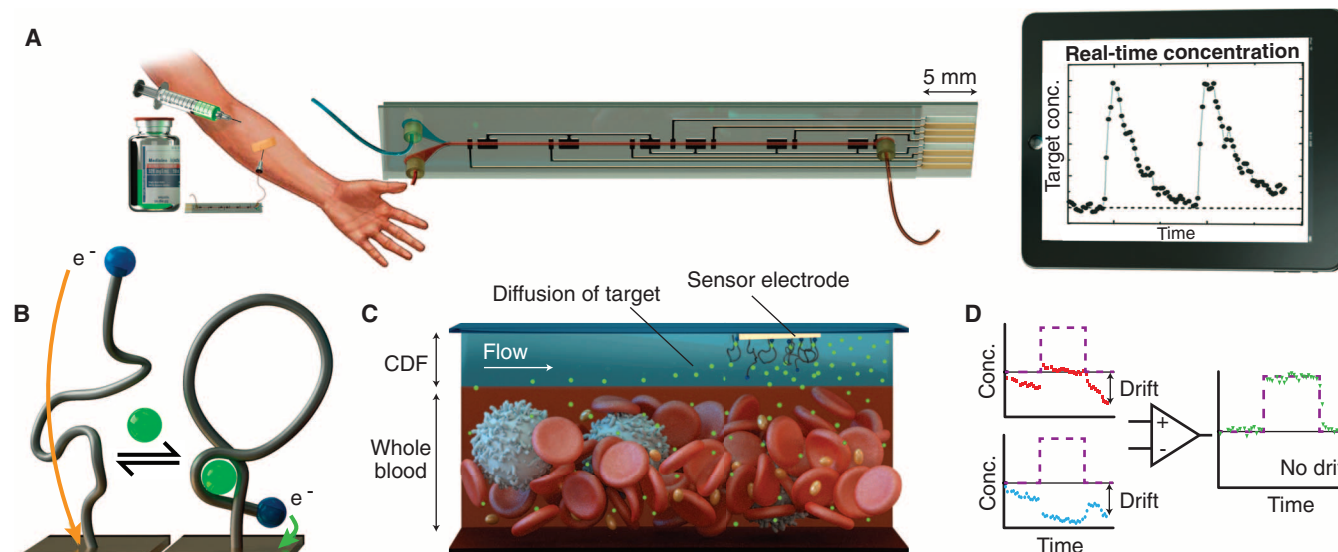


Fig. 1. MEDIC overview. MEDIC achieves real-time quantitative measurement of specific molecules in the blood of living animals. **(A)** Envisioned setup, where the MEDIC chip is connected to the patient's bloodstream to measure drug pharmacokinetics. **(B)** The aptamer probe is tethered to the gold electrode. Binding of target (green) induces a reversible conformational change in the probe, increasing the rate of electron transfer between an electrochemical redox reporter (blue) and a microfabricated electrode, yielding a measurable current change, shown in **(A)** as a func-

tion of time. **(C)** The continuous-flow diffusion filter (CDF), formed by vertically stacked laminar flow of buffer (blue) and blood (red), as shown in the microfluidic device in **(A)**, permits access to the target molecules while selectively excluding blood-borne interferences. **(D)** Signal-on (red) and signal-off (blue) both exhibit significant drift in response to a pulse of target (purple). Kinetic differential measurement (KDM; green) improves accuracy of real-time current measurements by minimizing drift and enhancing SNR.

variable patient pharmacokinetics and, when translated to the clinic, enable personalized and adaptable dosing for individual patients to drive optimal disease treatment.

RESULTS

MEDIC design overcomes challenges of continuous monitoring

MEDIC integrates multiple technological advances to overcome the challenges that have previously thwarted development of biosensors for continuous in vivo molecular detection. The central element of MEDIC is the electrochemical, aptamer-based sensor (13). This is a conformation-changing aptamer probe that is covalently attached via one terminus to an integrated electrode within the MEDIC device and modified at the other terminus with a redox reporter (Fig. 1B). Upon binding to its target molecule, the probe undergoes a reversible conformational rearrangement that modulates the redox current and generates an electrochemical signal. Detection is continuous and highly specific—only binding of the target triggers this conformational change, whereas nonspecific binding of interferences does not generate an electrochemical signal.

Within the MEDIC chip, the aptamer probes are protected by a continuous-flow diffusion filter (CDF), which prevents blood cells and other high-molecular weight interferences from physically occluding the sensor surface during operation (Fig. 1C). Finally, these are integrated with an electrochemical KDM technique that self-corrects for signal drift and enhances the SNR (Fig. 1D), enabling stable and quantitative detection over 4 hours of continuous operation.

Aptamer probes are sensitive and specific in space and time

For our initial tests, we designed a DOX-specific aptamer probe using a previously described DNA aptamer (14). We conjugated its 3' end to a methylene blue (MB) reporter and its 5' end to an alkane thiol for attachment to gold working electrodes within the MEDIC chip micro-channel (fig. S1). Target binding induces a conformational change in the aptamer that modulates electron transfer between MB and the electrode (Fig. 1B) (13). This modulation is expected to produce a readily measurable change in current at the MB reduction peak when the sensor is interrogated using square-wave voltammetry (SWV). The relative change in current (the signal gain) thus provides a direct measurement of DOX concentration.

To characterize the DOX probe's sensitivity, we fitted the probe's signal gain to a Langmuir isotherm and obtained an apparent equilibrium dissociation constant (K_d) of 824 ± 18 nM (mean $\pm$ SEM) (Fig. 2A). The sensor achieved a limit of detection (LOD) in buffer of 10 nM, with a dynamic range of 0.01 to 10 μ M, spanning the drug's therapeutic range (15). The sensor did not respond to 1000-fold greater concentrations of ifosfamide (Ifex), mesna, mitomycin C (MTC), dacarbazine (DTIC), or cisplatin (CDDP) (Fig. 2B)—agents commonly administered with DOX. The sensor also exhibited rapid kinetic response; after a 5-min pulse of 600 nM DOX, we measured $k_{on} = 3.0 \pm 0.35$ μ M $^{-1}$ min $^{-1}$ and $k_{off} = 1.35 \pm 0.05$ min $^{-1}$ (means $\pm$ SEM). The probe therefore reached 90% saturation within 45 s and then returned to within 10% of baseline by 100 s (Fig. 2C). Because the α -phase plasma clearance time of DOX in humans is between 6 and 26 min (16), this temporal resolution is sufficient for clinical applications.

The continuous diffusion filter prevents sensor fouling in human whole blood

Although aptamer-based electrochemical sensors perform well when challenged in undiluted blood serum (17), real-time measurement in whole blood has been thwarted to date by fouling from blood cells and high-molecular-weight clotting factors. We circumvented this problem with the CDF—a flowing, liquid-phase filter that exploits differences in diffusive transport (18). We established the CDF within the MEDIC chip by forming a vertically stacked laminar stream of isotonic buffer between the blood sample and electrodes (Fig. 1C).

Because of its high diffusivity ($1 \times 10^{-9} \text{ m}^2/\text{s}$) (19), DOX rapidly crossed the CDF, whereas larger, lower-diffusivity interferents, such as albumin, immunoglobulin (Ig), and fibrinogen, cannot reach the sensor surface during their transit time through the MEDIC chip. To optimize CDF performance, we modeled transport of DOX and several potential interferents, including human serum albumin (HSA), IgG, fibrinogen, and red blood cells. To do so, we created a finite-element model of the MEDIC channel to solve for velocity and concentration distributions. A CDF that is 125 μm high and with a total flow rate of 1 ml/hour yielded a Reynolds number of ~ 0.1 , enabling laminar flow stacking throughout the device. This is equivalent to a flow rate of 0.75 ml/hour for blood; under these conditions, sampling over the course of a full day would draw on only $\sim 0.3\%$ of the average human's blood volume.

We verified the flow pattern with confocal microscopy (fig. S2A), observing consistent results across multiple flow rate ratios (fig. S2B). To achieve optimal filtering performance, we adjusted CDF height and analyte residence time by controlling the total flow and ratio of the two flow rates. We calculated the average concentrations of DOX and interferents at the sensor surface (C_{surf}) as a function of position along the channel and normalized these to their input concentration (C_0) to yield the transport fraction, $\psi = C_{\text{surf}}/C_0$ (fig. S3). The optimal CDF yielded a transport fraction that was high for DOX ($\psi = 54\%$) and lower for interferents, including albumin, IgG, and fibrinogen ($\psi < 1\%$) (Fig. 2D). Sensor placement was optimal at 16 mm from the buffer-blood junction. Imaging before and after 20 min in flowing whole human blood confirmed that the CDF effectively prevented fouling of the sensor surface (Fig. 2E).

KDM eliminates drift during continuous blood monitoring

Accurate, long-term measurement requires maintaining sufficient SNR with minimal drift. Differential measurement strategies can counteract drift, but these typically require a physically separate reference probe to obtain a target-independent, common background signal that can be subtracted from the sensor signal. The reproducible fabrication of such a perfectly matched background-correction sensor is often challenging, rendering this approach problematic. To surmount this, we have developed KDM, which uses a single probe to obtain both measurements. KDM exploits the difference in charge transfer kinetics between the target-bound and unbound states of the probe. In the absence of target, the

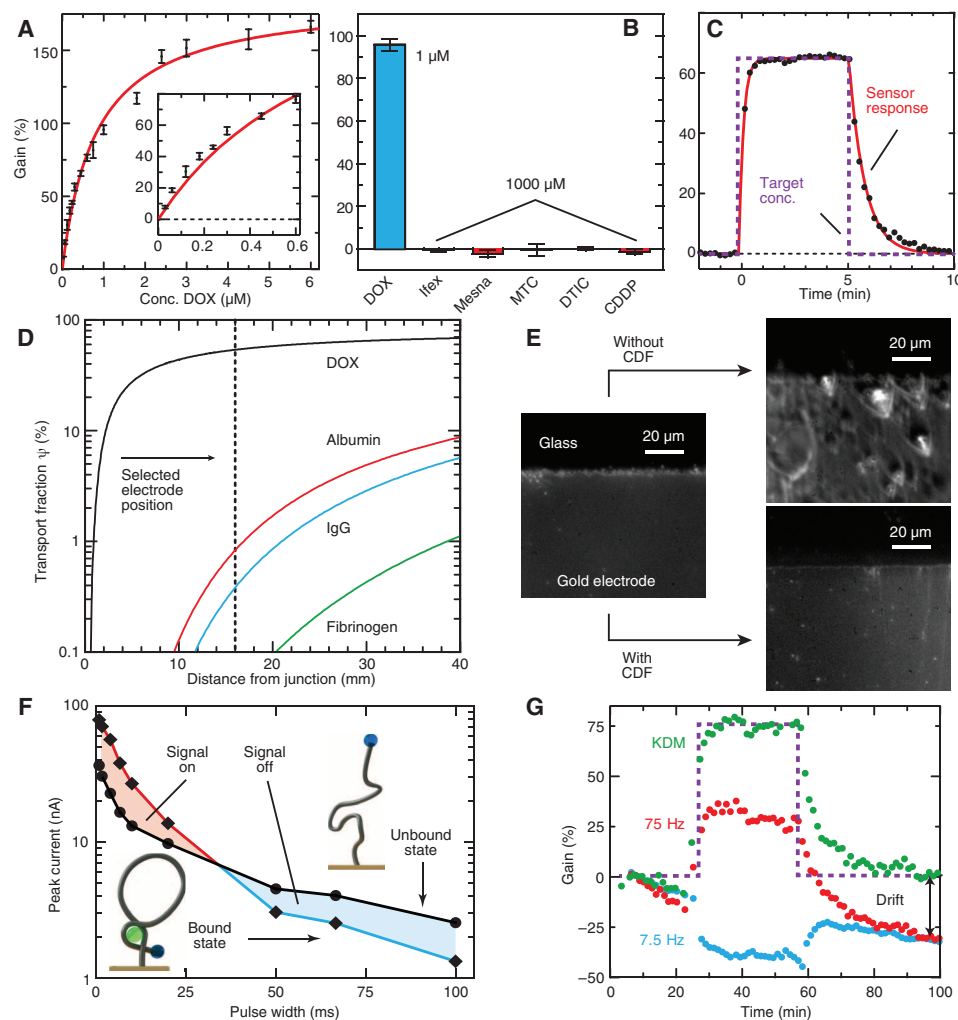


Fig. 2. Testing the core components of MEDIC. (A to C) DOX aptamer sensor characterization. (A) Dose response of the DOX aptamer probe to characterize LOD and dynamic range (mean $\pm$ SD, $n = 20$). (B) Demonstration of probe specificity based on lack of response to other chemotherapeutic agents (mean $\pm$ SD, $n = 15$). (C) Temporal response to DOX. (D) CDF design and performance. Numerical simulations of transport fraction for DOX and control proteins crossing the CDF. (E) Optical micrographs showed fouling in whole blood, as evidenced by the illuminated biomass collected on the electrode surface. (F) Mechanism and effectiveness of KDM. SWV pulse width is varied, and peak current is recorded in the unbound and DOX-bound states. Data are means ($n = 6$). In the unbound state (black line), MB is slowly reduced, yielding a gradual decay in current. Conversely, MB is rapidly reduced in the target-bound state (colored line). We can thus invert the response from “signal-on” to “signal-off” by changing the interrogation frequency. (G) Response to a DOX pulse (purple) at 75 and 7.5 Hz without KDM (red and blue lines, respectively). Applying KDM (green) minimized drift and increased signal amplitude.

unbound probe is unfolded and expanded; this results in a slow electron transfer rate, because the electrochemical reporter approaches the electrode surface only rarely (Fig. 2F) (20). The target-bound probe, in contrast, supports rapid electron transfer because it constrains the reporter near the electrode surface.

We interrogated the probe at multiple SWV frequencies by varying the pulse width ($\frac{1}{f}$) (fig. S4) to observe the rate of MB reduction. At short pulse widths, we observed higher current in the bound compared to the unbound state (Fig. 2F), as the faster kinetics allow more rapid reduction of MB. However, this rapid reduction results in depletion of MB, such that with longer pulse widths, we observed lower current in the bound state (Fig. 2F). This difference in electron transfer rate allowed us to obtain a signal-on response when we interrogated probes using high-frequency SWV, and a signal-off response when we interrogated at lower frequencies (fig. S5A). Critically, these two output signals exhibited matching responses to the background (fig. S5B); thus, we can differentially combine them to reduce drift. In response to a 2 μ M DOX pulse in whole blood over 90 min, KDM reduced the drift from ~ 30 to $<2\%$ (Fig. 2G). Moreover, because the two output signals differed in polarity, taking their difference yielded a $>300\%$ improvement in SNR (fig. S5C).

By combining the aptamer probes with these CDF and KDM techniques, MEDIC can achieve continuous, sensitive measurement of absolute analyte concentrations in a flowing stream of whole human blood for extended periods of operation (Fig. 3). To demonstrate this, we constructed a standard curve to relate concentration and differential gain (Fig. 3, inset). After pulsing DOX at 0.3, 0.5, 1, 2, and 4 μ M in whole human blood, MEDIC tracked concentrations with a stable baseline for 4 hours. During this time, the measured concentration differed from the known concentration on average by 0.06 μ M, verifying its ability to quantify unknown DOX concentrations continuously and in real time.

Real-time pharmacokinetic monitoring of multiple analytes in live animals

Having shown that MEDIC can reliably operate in human whole blood *in vitro*, we next performed real-time DOX measurements using live rats. Briefly, we injected DOX intravenously into anesthetized Sprague-Dawley rats while continuously drawing blood into the chip using an indwelling catheter. We dosed a rat with increasing DOX concentrations (0 to 2 mg/m²) over 4.5 hours (Fig. 4A). Vehicle-only injection (negative control) yielded no observable signal change. In contrast, the lowest DOX dose (0.1 mg/m²) resulted in a peak *in vivo* concentration of 0.13 μ M, whereas doses of 0.2, 0.6, and 2 mg/m² resulted in peak concentrations of 0.3, 1.0, and 2.5 μ M, respectively—a therapeutically relevant range for human dosing (15). A final measurement made on blood collected before dosing confirmed minimal drift during the experiment (0.1 μ M) (Fig. 4A). We observed a signal in rats injected with the chemotherapeutic drug cocktails DMAP (dacarbazine, MTC, DOX, and cisplatin) and AIM (DOX, ifosfamide, and mesna) when these cocktails contained DOX, but not when DOX was excluded (Fig. 4, B and C).

This capability to continuously measure *in vivo* DOX concentrations enabled us to obtain key pharmacokinetic parameters for individual animals in real time. For example, after administering two DOX injections (1 mg/m²) an hour apart to the same rat, we reproducibly measured equivalent peak concentrations (1.17 and 1.16 μ M) and α -phase half-life values ($t_{1/2}^{\alpha} = 10.7 \pm 0.5$ and 10.8 ± 1 min; means $\pm$ SEM) (Fig. 4D). These values agree with previously published values for intravenously injected DOX in rats (21). Highlighting the potential importance of such “personalized” pharmacokinetics, we observed substantial variability in $t_{1/2}^{\alpha}$ when we performed these measurements in nine rats (fig. S6). Indeed, this variability held even when we normalized the dose for each animal by body surface area, the current gold standard for DOX dosing (Pearson correlation coefficient = -0.73).

MEDIC can be readily reconfigured to measure different target molecules by exchanging its aptamer probe in a modular manner. As an exemplar, we exchanged our DOX probe for one that supports continuous detection of the antibiotic kanamycin (22). After obtaining a calibration curve as described above (fig. S7), we serially injected rats with kanamycin doses of 32, 64, and 96 mg/kg, followed by a baseline control. We observed peak concentrations of 0.45, 0.85, and 1.6 mM, respectively (Fig. 4E), and measured $t_{1/2}^{\alpha}$ of 7.2, 10, and 9 min, in agreement with previously reported values for kanamycin (23). Unlike DOX, however, kanamycin concentrations did not return to baseline because much of the clearance of this drug occurs in the β phase ($t_{1/2}^{\beta} \approx 2$ to 3 hours) (24), which is considerably longer than our dosing interval (40 min).

DISCUSSION

We present a real-time biosensor in a miniaturized, disposable chip format that can continuously measure the concentration of a wide range of circulating molecules in living animals with excellent precision and subminute temporal resolution. We achieved direct measurement of therapeutic concentrations of DOX in human whole blood even when co-administered with a 1000-fold excess of other chemotherapeutic agents. This illustrates that the MEDIC device is highly sensitive and specific. Experiments in rodents demonstrated that MEDIC is effective at discriminating individual differences in drug pharmacokinetics. Variability in pharmacokinetic parameters has been reported

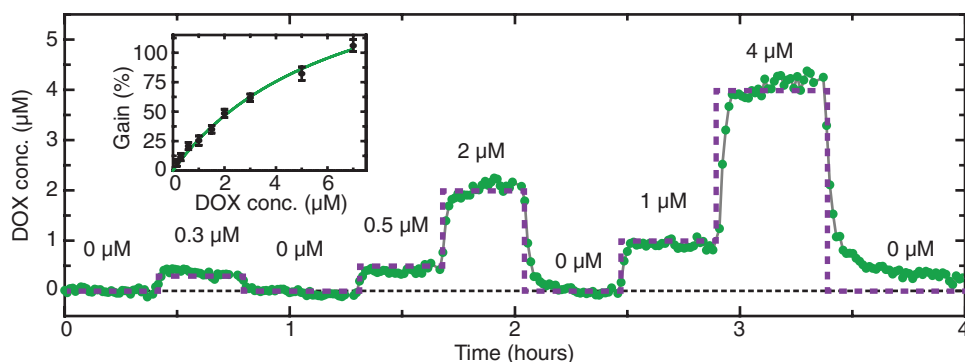


Fig. 3. Real-time measurement of DOX *in vitro* in human whole blood. Continuous, real-time measurement of DOX in human whole blood (green) relative to actual concentrations (purple) over the course of 4 hours. Blood flow rate = 0.75 ml/hour; buffer flow rate = 0.25 ml/hour. Inset shows a standard curve relating KDM signal to DOX concentration. Data are means $\pm$ SD ($n = 20$).

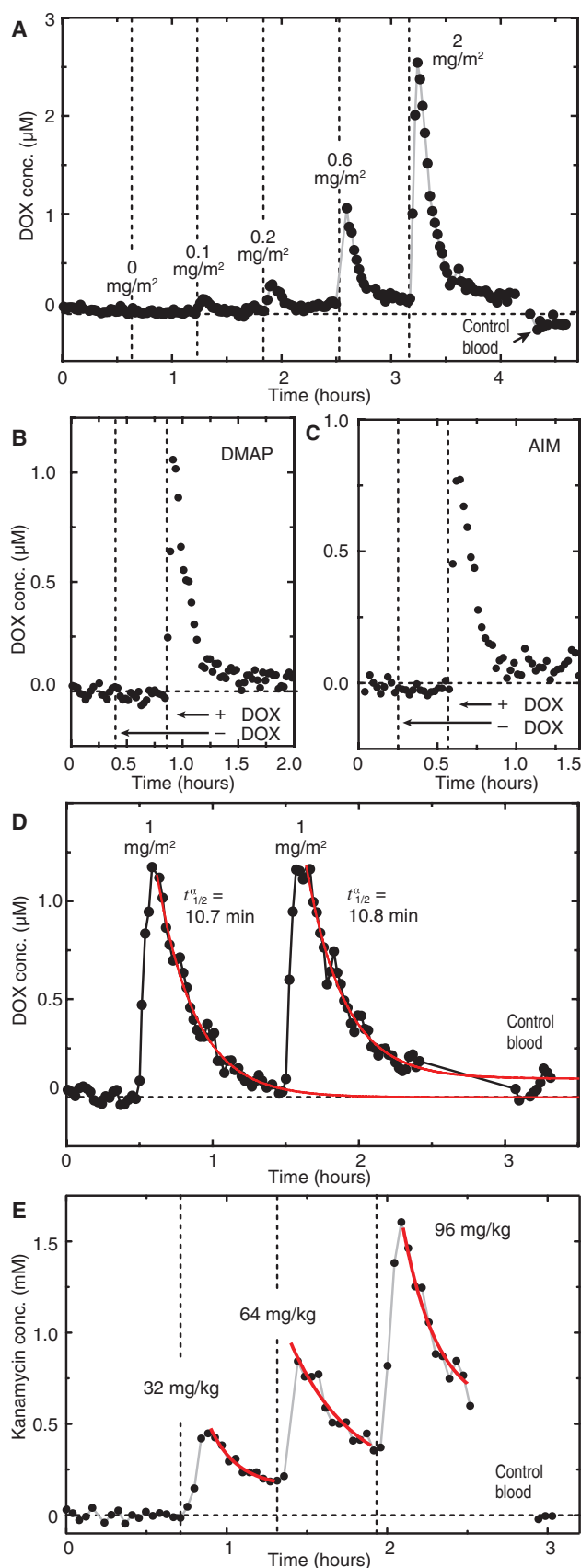


Fig. 4. Real-time measurement of drugs in the blood of live rats. (A) MEDIC measurement of changing in vivo DOX concentrations over several hours. (B and C) Sensor specificity demonstrated in vivo in rats. We subjected rats to chemotherapy cocktails DMAP (B) and AIM (C) in the absence or presence of DOX. (D) Peak concentrations and $t_{1/2}^a$ after two intravenous injections of DOX into a rat. (E) MEDIC analysis with the aptamer probe substituted to enable continuous real-time measurement of kanamycin. Control blood contained no drug. Measurements were performed at the end of the experiment by switching the blood draw from the animal to a tube containing a sample of the animal's blood taken at the beginning of the experiment, before injection of the drug. The gap between these measurements represents the time required to transition the intake tube from the animal to this control sample. Each graph corresponds to a single animal, and each data point corresponds to a single KDM signal.

for DOX in humans (25), which can result in markedly different patient outcomes (26). This offers strong evidence for this platform's potential clinical utility in designing patient-appropriate dosing regimens. Finally, the modular architecture of MEDIC means that it can be adapted to a wide range of target molecules simply by exchanging the aptamer probes, as demonstrated by our follow-up real-time measurements of kanamycin in live rats.

MEDIC's real-time measurement capabilities could establish a new paradigm in therapeutic drug monitoring, with immediate applications in acute care. As reported in the literature (27), drug dosing strategies based on pharmacokinetic models using physiological parameters such as sex, mass, or body surface area often fail to capture clinically important inter- and inpatient variability. This may, in part, account for the inconsistencies often observed in response to therapy, wherein patients receiving similar doses of DOX, for example, exhibit markedly different toxic and therapeutic responses (12, 28). Accordingly, individualized drug dosing based on therapeutic drug monitoring has led to considerable improvements in patient outcomes, especially for drugs with narrow therapeutic ranges (29). Unfortunately, current monitoring strategies use methods such as chromatography, immunoassays, or mass spectrometry to measure drug levels, requiring significant sample handling and processing that can delay availability of results for days (30). This lag time is a long-standing problem in acute clinical settings such as emergency medicine, surgery, and treatment of infectious disease, where immediate response to rapidly changing conditions is critical (31). By tracking patient pharmacokinetics in real-time, MEDIC could give clinicians the capacity to provide individualized and optimal dose control at the point of care.

Although we have demonstrated a potential solution to a long-standing problem, there are several limitations in this study. First, MEDIC was configured to measure the pharmacologically active free form of the drug in whole blood. This is valuable for monitoring a range of drugs including chemotherapeutics, anti-infective agents, immunosuppressants, and anticonvulsants (32). However, for many drugs, it is important to measure their metabolites or drug-protein complexes. To detect these molecules, it will be important to develop appropriate aptamer probes that can specifically distinguish and measure the free drug, metabolites, and complexes. Fortunately, aptamers have been discovered for a broad spectrum of biological molecules (33), and by using advanced selection techniques, aptamers with exquisite specificity to closely related analogs can be generated (34). Second, we have presently demonstrated continuous MEDIC operation with stable performance over multiple hours. Although we believe that

this range of continuous operation is sufficient for many acute clinical applications, the capacity for much longer-term operation (for example, days and weeks) could prove highly valuable in chronic care applications, and improved device architecture to support substantially longer operation is a subject of ongoing work.

Although we tested the performance of the device with whole human blood, we have not yet tested MEDIC directly connected to human patients. Such transition will require extensive testing to satisfy regulatory requirements. Specifically, comparative studies will be needed to determine the absolute accuracy of our MEDIC system with respect to standard reference values over time, and to ascertain the frequency with which the disposable sensor must be replaced. One advantage of our device from a regulatory perspective is that it can analyze minimal blood volumes; as such, we do not anticipate the need to recirculate blood back to patients after testing. We initially envision the use of MEDIC as a replacement for standard periodic measurement in hospitals and other clinical settings. It will therefore be essential for us to demonstrate improved patient outcomes and to make the operation of the system sufficiently facile for nurses and other caregivers. The entry of continuous glucose monitoring (CGM) technology into the clinical market over the past decade offers an invaluable precedent and will be used as a model for us to translate our technology.

We also foresee numerous opportunities for extending the capabilities of MEDIC, for example, simultaneously measuring the drug being administered as well as the biomarkers indicative of the patient's response to that drug by integrating multiple probes into one device (35). This would facilitate a closed feedback loop between the drug and the patient's response, which can then be adjusted to continuously deliver an optimal therapeutic dose in real time. For example, optimal dosing of the immunosuppressant cyclosporine for patients undergoing organ transplantation is notoriously difficult because of inter- and inpatient variability (36). Appropriate dosing is crucial because the effects of the drug are proportional to its concentration (37), but cyclosporine dose has also been correlated with levels of proinflammatory chemokines such as CXCL9 (38, 39), which are associated with nephropathy, acute graft rejection, and graft failure (40). Thus, the capacity to continuously measure both CXCL9 and cyclosporine using MEDIC could enable continuous, closed-loop delivery of optimal cyclosporine doses to enhance therapeutic outcome for individual patients in real time (41, 42). Such adaptive dosing technology could also enable the expanded use of drugs with narrow therapeutic indices. In this way, just as real-time CGMs have revolutionized diabetes care and contributed to the ongoing development of an "artificial pancreas" via closed-loop control of insulin (43), we believe that MEDIC could offer a general framework for achieving closed-loop control of numerous different *in vivo* biological processes, thereby yielding more effective treatments for a broad range of medical conditions.

MATERIALS AND METHODS

Study design

Our study consisted of three major components: *in vitro* characterization with buffers, human whole-blood testing, and live animal testing. The objective was to demonstrate proof-of-concept capability to monitor drugs directly from the blood of living subjects in real time. Characterization in buffer was required to demonstrate function of the aptamer probe, including its sensitivity, specificity, and temporal response. Human

whole-blood samples were used to assess the capacity of the CDF and KDM to support real-time measurement of target for a period of at least 4 hours, and compared to measurement without these components. Our animal study protocol, *In Vivo* Small Molecule Detection, was approved by the University of California, Santa Barbara (UCSB) Institutional Animal Care and Use Committee and assigned protocol number 824. Measurements were taken once animal surgeries were stabilized, and concluded with euthanasia of each animal. Blood samples and individual animals were selected randomly for each experiment. Blinding was not applicable to this study, and none of the experiments were blinded. Replication conditions for each experiment are defined and described in Results.

Device fabrication

Two Borofloat glass wafers (top and bottom; Mark Optics Inc.), each 10 cm in diameter and 500 μm thick, were cleaned by sequential immersion in acetone, isopropanol, and deionized water. Gold working, counter, and reference electrodes were then patterned onto the top wafer at 8-mm intervals photolithographically and electron beam-evaporated (VES 2550, Temescal) to a thickness of 200 nm on 20-nm titanium adhesion layers. Subsequently, a CNC mill (Flashcut CNC) with 1.1-mm diamond bit (Triple Ripple, Abrasive Technology) was used to drill fluidic vias (two inlets, one outlet) in the top wafer. The top and bottom wafers were then diced (DISCO) into device pieces with dimensions of 58 mm $\times$ 11 mm and 53 mm $\times$ 11 mm, respectively.

The 500- μm -wide top (buffer) and bottom (blood) flow channels were both cut from a 250- μm -thick polydimethylsiloxane sheet (BISCO Silicones, Rogers Corp.) using a cutting plotter (CE5000-60, Graphtec). The buffer channel was first bonded to the diced electrode glass substrate via 10-s corona-ozone treatment (BD-20AC, Electro-Technic Products Inc.). The blood channel was bonded to the buffer channel, and then to the bottom glass substrate, each with the same ozone treatment. Alignment was performed under a standard inverted microscope via an x - y - z - θ stage (Newport Corp.) and vacuum chuck. Fluidic port connectors (LabSmith) were glued onto the device with 5-min epoxy (ITW Devcon), readying the chip for probe immobilization.

Fluidic instrumentation

All flow to the device was controlled via a syringe pump (PhD 2000, Harvard Apparatus). The MEDIC input port was connected to an intravenous cannula for animal studies or multiport valve (Upchurch Scientific) for *in vitro* studies. The valve was used to select samples bearing different target concentrations. A 3-ml syringe loaded with 1 $\times$ SSC was placed in a pump and connected to the buffer port on the MEDIC device via a 30-cm length of Tygon (Saint-Gobain Performance Plastics) tubing, with 2.29-mm outer diameter (OD) and 0.76-mm inner diameter (ID). The MEDIC device output port was connected to a primed but otherwise empty 10-ml "waste" syringe placed in a second pump via $1/16$ -inch OD and 0.5-mm ID Teflon tubing (Thomson Instrument Co.). To monitor flow rates in real time, we used a flow meter (Mitos Flow Rate Sensor, Dolomite Microfluidics) in-line between the output port and syringe pump. The buffer layer was established by engaging the buffer pump at 0.25 ml/hour. Simultaneously, sample was continuously drawn into the device by engaging the waste pump at 1 ml/hour.

Aptamer probe preparation and immobilization

The aptamer probes were synthesized by Biosearch Technologies with the following sequences: DOX-28: 5'-HS-(CH₂)₆-ACCATCTGTG-TAAGGGGTAAGGGGTGGT-MB-3'; kanamycin: 5'-HS-(CH₂)₆-

GGGACTTGGTTTAGGTAATGAGTCCC-MB-3'. DOX-28 is a truncated form of the Wochner 41-nucleotide anthracycline aptamer (14). We selected a region of the aptamer suspected to support a hairpin structure, with an apparent active binding site in the base. We engineered the stem to enable effective signal transduction through stabilization of the folded state. The kanamycin probe was adapted from (22). Each probe was thiolated at the 5' end to facilitate self-assembly on the gold working electrodes, and conjugated with an MB redox label at the 3' end to enable target binding-induced charge transfer modulation. Gold electrode cleaning and probe immobilization methods were adapted from (17).

To measure aptamer affinity, we obtained a dose-response curve by subjecting the probe to DOX (Sigma-Aldrich) concentrations ranging from 50 nM to 10 μ M in 1 $\times$ SSC at a flow rate of 1 ml/hour. At each concentration, the signal was permitted to equilibrate, and then 10 subsequent readings were obtained from two separate electrodes and averaged as the reported values. The same procedure was applied for specificity characterization, where DOX was injected at 1 μ M, whereas off-target reagents MTC, Ifex, mesna, DTIC, and CDDP (Sigma-Aldrich) were injected at 1 mM concentrations. Kinetic measurements were performed in a reaction-limited regime by flowing target-free buffer at 10 ml/hour for 5 min to obtain a stable baseline, followed by a 5-min 1 μ M DOX pulse, and then back to buffer. Voltammograms were taken in immediate succession to provide maximal temporal resolution. Dose-response curves were obtained by fitting data to a one-site binding model, and kinetic-response curves were obtained by fitting data to a one-phase association and dissociation model (GraphPad Prism version 5.00C for Mac OS X, GraphPad Software).

LOD was calculated as the concentration that gives a signal three baseline SDs (s_{baseline}) above zero:

$$\text{LOD} = K_d \frac{\frac{3s_{\text{baseline}}}{D_{\text{max}}}}{1 - \frac{3s_{\text{baseline}}}{D_{\text{max}}}}$$

To determine the dynamic range, we calculated the maximum detectable concentration (MDC) as the concentration where the average is 3 SDs less than maximum signal. Here, s_{max} is the average SD of the four largest concentrations used:

$$\text{MDC} = K_d \frac{\frac{D_{\text{max}} - 3s_{\text{max}}}{D_{\text{max}}}}{1 - \frac{(D_{\text{max}} - 3s_{\text{max}})}{D_{\text{max}}}}$$

Voltammetry

Electrochemical measurements were conducted with an electrochemical analyzer (730B, CH Instruments). The chip was connected via an eight-pin card-edge connector (Molex) and subjected to SWV scans. Working electrodes were scanned in continuous succession with an average scan period of 8 s. To compare the potential difference between the patterned reference electrode and a standard Ag/AgCl electrode, we submerged the channel into a flask that contained buffer and used an Ag/AgCl rod electrode with porous Teflon tip (CH Instruments). MB redox peak was typically observed at about -260 mV (with respect to Ag/AgCl reference) or about -110 mV (with respect to the patterned reference electrode). A potential range of -50 to -450 mV (with respect to Ag/AgCl reference) was scanned to reliably capture

the entire MB reduction peak. To determine the optimal frequency for KDM, we performed frequency-sweep scans from 5 to 800 Hz, with an amplitude of 25 mV. A custom peak-fitting script was created to fit the SWV measurements with a Gaussian curve on a hyperbolic baseline. Peak currents were then normalized to a baseline peak current to generate signal gain. Unless otherwise noted, all reported gains were obtained via KDM with the difference divided by the average of gains from 7.5- and 75-Hz signals. To demonstrate sensing with a handheld electrochemical analyzer, we used the PalmSens EmStat2 (Palm Instruments BV). We observed a comparable effective signal; to account for instrument differences, KDM was performed with constituent frequencies of 150 and 5 Hz, and a separate dose-response curve was obtained.

CDF characterization

COMSOL Multiphysics v4.2a was used to simulate target and non-target diffusion in the microchannel in three dimensions via laminar flow and transport of diluted species modules. To simplify the simulation geometry, we focused on the straight portion of the channel from the junction of the two vertically stacked flows to the outlet region. On the basis of an expected operational sample-to-buffer throughput ratio of 3:1, the domain depths were assigned to 400 and 100 μ m, respectively. Input flow rates were defined at 0.75 and 0.25 ml/hour for buffer and sample, respectively, with open pressure defined at the output. Buffer was represented as water, whereas the blood sample was approximated as water with density increased to 1060 kg/m³ and viscosity increased to 4 mPa·s. A diffusion constant of 1×10^{-9} m²/s was assumed for DOX (19). The diffusivities of HSA, IgG, and fibrinogen—blood protein interferents associated with biofouling—were estimated as 5×10^{-11} , 4×10^{-11} , and 2×10^{-11} m²/s, respectively (44–46). Analytes were supplied in the sample stream at a concentration of 1 μ M at the sample input and convected according to the prior-solved velocity field, and reported concentrations were normalized by this value for the respective species. Solving for the velocity field and then the convection diffusion equation yields the concentration field throughout the channel. An x - y cross section was taken at the top of the channel in the plane of the electrodes (fig. S3A), scaled at 20% in x with respect to y . This cross section confirmed an expected transport fraction in excess of ~50% of input at a point 20 mm from the junction, with parabolic isolines. Examination of y - z sections along the channel (fig. S3B) showed diffusion of DOX from the target-rich flow stream to the initially pure buffer stream.

All confocal imaging data were obtained via FluoView 1000 MPE (Olympus) with a 10 $\times$ objective. To obtain unobstructed views of the channel, we fabricated devices exclusively for imaging that were identical to normal devices save the absence of electrodes. These were mounted upside-down on a microscope slide clipped to the microscope stage to prevent the connectors from occluding the view of the channel. With the device mounted upside-down, the buffer and blood inputs were switched to enable normal operation with blood on the bottom and buffer on the top. The motorized stage enabled digital encoding of location data. The x - y origin was set at the center of the channel junction for a given device. All location data were thus derived from the stage readout with respect to junction center. All image depth data were likewise derived from the readout of the motorized nosepiece actuating in the z axis. To image the buffer and blood streams, we respectively added fluorescein and rhodamine WT fluorescent dyes (Bright Dyes) to each at a 1:10,000 dilution from stock. The rhodamine and fluorescein dyes were excited with 559- and

473-nm lasers, respectively. To negate channel crosstalk, we collected all images via sequential illumination. To demonstrate flow stacking in the absence of diffusion, we increased the flow rates of the buffer and blood streams 10-fold to 2.5 and 7.5 ml/hour, respectively, because small-molecule dye diffusion was readily observed under normal operating conditions.

KDM characterization

The rate of charge transfer from our redox-labeled probe is a function of the applied voltage, probe flexibility, and redox reporter location. At a fixed voltage,

$$I = -F \frac{d[\text{MB}]}{dt} = F(k_1[\text{MB}] - k_{-1}[\text{leucoMB}])$$

where F is Faraday's constant, $[\text{MB}]$ is the concentration of methylene blue, $[\text{leucoMB}]$ is the concentration of reduced leucomethylene blue, and k_1 and k_{-1} are the forward and reverse reaction rates, respectively. In the unbound probe conformation, the kinetics were slow and MB was only gradually reduced to leucoMB. However, in the bound conformation, the kinetics were fast and MB was reduced rapidly.

The KDM signal (D) was obtained by taking the difference of the signal-on (G_{on}) and signal-off (G_{off}) and dividing by the average as follows:

$$D = \frac{G_{\text{on}} - G_{\text{off}}}{\frac{(G_{\text{on}} + G_{\text{off}})}{2}}$$

Optimal KDM was obtained by selecting G_{on} and G_{off} at frequencies with well-matched drift. This was determined empirically by examining drift over a range of frequencies.

We defined SNR as follows:

$$\text{SNR} = \frac{\overline{D_{\text{target}}}}{\sqrt{s_{\text{baseline}}^2 + s_{\text{target}}^2}}$$

where $\overline{D_{\text{target}}}$ is the average signal in the presence of target and s_{target} is the SD.

Human whole blood

We obtained male human whole blood with sodium citrate anticoagulant (Bioreclamation LLC) within about 1 week of the date it was drawn from the donor. Whole blood was refrigerated at 4°C until use. Before use, blood was warmed to room temperature and then passed through a 40-μm pore cell strainer (Thermo Fisher Scientific) to remove or break up any large aggregates while letting blood constituents pass through. Concentrated target drug was then doped to the desired dose. Whole blood was otherwise unmodified.

Live animal studies

All rats used in this work were Sprague-Dawley males ($n = 9$) from Charles River Laboratories. To safely mount the device and catheters, we sedated rats throughout the duration of the experiment via isoflurane gas (1 to 4%) and ketamine/xylazine (56.25 and 7.5 mg/kg, respectively). Animals were placed dorsal side down on a heating pad. The right jugular vein was isolated and catheterized (11 cm long, 0.64-mm OD, 0.3-mm ID; Dow Corning) to provide an intravenous injection port. We performed an initial injection of 0.4-ml heparin solution (USP, 1000 U/ml), followed by periodic injections of 0.1 ml every 40 min

to prevent clot formation in the catheters. The left jugular vein was isolated and cannulated (Insyte Autoguard Shielded IV Catheter, Becton-Dickinson). A 0.3-ml predrug injection control blood sample was taken and set aside for the duration of the experiment. The end of a 10-cm length of Tygon tubing (2.29-mm OD and 0.76-mm ID) was inserted into the cannula to connect the intravenous blood source to the blood port of the MEDIC device. The remaining fluidic instrumentation was configured as described in the previous section. Target concentrations were adjusted for a constant 0.3-ml injection volume and injected via the input catheter in a 30-s bolus at >30-min intervals. Upon completion of each experiment, subjects were euthanized via intravenous Euthasol (Virbac Animal Health) injection. The MEDIC input tube was then redirected to the control blood to obtain postexperiment readings of zero-target blood samples. Observed concentrations were derived from a dose-response calibration curve obtained a priori (fig. S7). Dosing was normalized to the animal's body surface area.

SUPPLEMENTARY MATERIALS

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Fig. S1. Device architecture and fabrication.

Fig. S2. Confocal analysis of human whole blood in MEDIC channel.

Fig. S3. Finite element model of diffusive transport of target in the MEDIC channel.

Fig. S4. Schematic illustration of SWV.

Fig. S5. KDM characterization.

Fig. S6. DOX α -phase half-life measurement in a cohort of rats.

Fig. S7. Analyte dose responses in human whole blood in MEDIC chip.

REFERENCES AND NOTES

1. M. A. Hamburg, F. S. Collins, The path to personalized medicine. *N. Engl. J. Med.* **363**, 301–304 (2010).
2. D. M. Rissin, C. W. Kan, T. G. Campbell, S. C. Howes, D. R. Fournier, L. Song, T. Piech, P. P. Patel, L. Chang, A. J. Rivnak, E. P. Ferrell, J. D. Randall, G. K. Provuncher, D. R. Walt, D. C. Duffy, Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. *Nat. Biotechnol.* **28**, 595–599 (2010).
3. F. Patolsky, G. Zheng, C. M. Lieber, Fabrication of silicon nanowire devices for ultra-sensitive, label-free, real-time detection of biological and chemical species. *Nat. Protoc.* **1**, 1711–1724 (2006).
4. D. A. Baker, D. A. Gough, A continuous, implantable lactate sensor. *Anal. Chem.* **67**, 1536–1540 (1995).
5. G. S. Wilson, Y. Hu, Enzyme-based biosensors for in vivo measurements. *Chem. Rev.* **100**, 2693–2704 (2000).
6. T. Keller, T. Zeller, D. Peetz, S. Tzikas, A. Roth, E. Czyz, C. Bickel, S. Baldus, A. Warnholtz, M. Fröhlich, C. R. Sinning, M. S. Eleftheriadis, P. S. Wild, R. B. Schnabel, E. Lubos, N. Jachmann, S. Genth-Zotz, F. Post, V. Nicaud, L. Tiret, K. J. Lackner, T. F. Münzel, S. Blankenberg, Sensitive troponin I assay in early diagnosis of acute myocardial infarction. *N. Engl. J. Med.* **361**, 868–877 (2009).
7. J. W. Bauer, M. Petri, F. M. Batliwalla, T. Koeuth, J. Wilson, C. Slattery, A. Panoskaltis-Mortari, P. K. Gregersen, T. W. Behrens, E. C. Baechler, Interferon-regulated chemokines as biomarkers of systemic lupus erythematosus disease activity: A validation study. *Arthritis Rheum.* **60**, 3098–3107 (2009).
8. M. Das Thakur, F. Salangsang, A. S. Landman, W. R. Sellers, N. K. Pryer, M. P. Levesque, R. Dummer, M. McMahon, D. D. Stuart, Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature* **494**, 251–255 (2013).
9. P. Yager, T. Edwards, E. Fu, K. Helton, K. Nelson, M. R. Tam, B. H. Weigl, Microfluidic diagnostic technologies for global public health. *Nature* **442**, 412–418 (2006).
10. R. S. Gaster, D. A. Hall, C. H. Nielsen, S. J. Osterfeld, H. Yu, K. E. Mach, R. J. Wilson, B. Murmann, J. C. Liao, S. S. Gambhir, S. X. Wang, Matrix-insensitive protein assays push the limits of biosensors in medicine. *Nat. Med.* **15**, 1327–1332 (2009).
11. K. W. Plaxco, H. T. Soh, Switch-based biosensors: A new approach towards real-time, in vivo molecular detection. *Trends Biotechnol.* **29**, 1–5 (2011).
12. B. Desoize, J. Robert, Individual dose adaptation of anticancer drugs. *Eur. J. Cancer* **30A**, 844–851 (1994).

13. Y. Xiao, A. A. Lubin, A. J. Heeger, K. W. Plaxco, Label-free electronic detection of thrombin in blood serum by using an aptamer-based sensor. *Angew. Chem. Int. Ed. Engl.* **44**, 5456–5459 (2005).
14. A. Wochner, M. Menger, D. Orgel, B. Cech, M. Rimmele, V. A. Erdmann, J. Glöckler, A DNA aptamer with high affinity and specificity for therapeutic anthracyclines. *Anal. Biochem.* **373**, 34–42 (2008).
15. R. F. Greene, J. M. Collins, J. F. Jenkins, J. L. Speyer, C. E. Myers, Plasma pharmacokinetics of Adriamycin and adriamycinol: Implications for the design of in vitro experiments and treatment protocols. *Cancer Res.* **43**, 3417–3421 (1983).
16. C. Camaggi, R. Comparsi, E. Strocchi, F. Testoni, B. Angelelli, F. Pannuti, Epirubicin and doxorubicin comparative metabolism and pharmacokinetics. A cross-over study. *Cancer Chemother. Pharmacol.* **21**, 221–228 (1988).
17. J. S. Swensen, Y. Xiao, B. S. Ferguson, A. A. Lubin, R. Y. Lai, A. J. Heeger, K. W. Plaxco, H. T. Soh, Continuous, real-time monitoring of cocaine in undiluted blood serum via a microfluidic, electrochemical aptamer-based sensor. *J. Am. Chem. Soc.* **131**, 4262–4266 (2009).
18. J. P. Brody, P. Yager, Diffusion-based extraction in a microfabricated device. *Sens. Actuators A Phys.* **58**, 13–18 (1997).
19. S. Eikenberry, R. tumor cord model for doxorubicin delivery and dose optimization in solid tumors. *Theor. Biol. Med. Model.* **6**, 16 (2009).
20. T. Uzawa, R. R. Cheng, R. J. White, D. E. Makarov, K. W. Plaxco, A mechanistic study of electron transfer from the distal termini of electrode-bound, single-stranded DNAs. *J. Am. Chem. Soc.* **132**, 16120–16126 (2010).
21. Y. Ueda, K. Munechika, A. Kikukawa, Y. Kanoh, K. Yamanouchi, K. Yokoyama, Comparison of efficacy, toxicity and pharmacokinetics of free adriamycin and adriamycin linked to oxidized dextran in rats. *Chem. Pharm. Bull.* **37**, 1639–1641 (1989).
22. A. A. Rowe, E. A. Miller, K. W. Plaxco, Reagentless measurement of aminoglycoside antibiotics in blood serum via an electrochemical, ribonucleic acid aptamer-based biosensor. *Anal. Chem.* **82**, 7090–7095 (2010).
23. M. H. Al-Nazawi, A. M. Homeida, Comparative pharmacokinetic studies of kanamycin in camels, sheep and goats. *Sci. J. King Faisal Univ.* **10**, 105–113 (2008).
24. D. McGregor, in *Kirk-Othmer Encyclopedia of Chemical Technology* (John Wiley & Sons Inc., New York, 2000).
25. N. A. Dobbs, C. J. Twelves, H. Gillies, C. A. James, P. G. Harper, R. D. Rubens, Gender affects doxorubicin pharmacokinetics in patients with normal liver biochemistry. *Cancer Chemother. Pharmacol.* **36**, 473–476 (1995).
26. A. Elis, M. Lishner, S. Walker, D. Atias, A. Korenberg, G. Koren, Doxorubicin in lymphoma: Association between pharmacokinetic variability and clinical response. *Ther. Drug Monit.* **32**, 50–52 (2010).
27. F. Khalil, S. Læer, Physiologically based pharmacokinetic modeling: Methodology, applications, and limitations with a focus on its role in pediatric drug development. *J. Biomed. Biotechnol.* **2011**, 907461 (2011).
28. D. M. Bach, J. A. Straseski, W. Clarke, Therapeutic drug monitoring in cancer chemotherapy. *Bioanalysis* **2**, 863–879 (2010).
29. W. E. Evans, M. V. Relling, J. H. Rodman, W. R. Crom, J. M. Boyett, C. H. Pui, Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. *N. Engl. J. Med.* **338**, 499–505 (1998).
30. R. DiFrancesco, S. Rosenkranz, A. L. Mukherjee, L. M. Demeter, H. Jiang, R. DiCenzo, C. Dykes, A. Rinehart, M. Albrecht, G. D. Morse, Quality assessment for therapeutic drug monitoring in AIDS Clinical Trials Group (ACTG 5146): A multicenter clinical trial. *Ther. Drug Monit.* **32**, 458–466 (2010).
31. A. Boyer, D. Gruson, S. Bouchet, B. Clouzeau, B. Hoang-Nam, F. Vargas, H. Gilles, M. Molimard, A. M. Rogues, N. Moore, Aminoglycosides in septic shock: An overview, with specific consideration given to their nephrotoxic risk. *Drug Saf.* **36**, 217–230 (2013).
32. D. Gonzalez, S. Schmidt, H. Derendorf, Importance of relating efficacy measures to unbound drug concentrations for anti-infective agents. *Clin. Microbiol. Rev.* **26**, 274–288 (2013).
33. A. D. Keefe, S. Pai, A. Ellington, Aptamers as therapeutics. *Nat. Rev. Drug Discov.* **9**, 537–550 (2010).
34. R. D. Jenison, S. C. Gill, A. Pardi, B. Polisky, High-resolution molecular discrimination by RNA. *Science* **263**, 1425–1429 (1994).
35. E. Pavlovic, R. Y. Lai, T. T. Wu, B. S. Ferguson, R. Sun, K. W. Plaxco, H. T. Soh, Microfluidic device architecture for electrochemical patterning and detection of multiple DNA sequences. *Langmuir* **24**, 1102–1107 (2008).
36. C. Y. Chiang, H. G. Schneider, B. Levvey, L. Mitchell, G. I. Snell, Tacrolimus level variability is a novel measure associated with increased acute rejection in lung transplant (LTx) recipients. *J. Heart Lung Transplant.* **32**, S170 (2013).
37. P. F. Halloran, Immunosuppressive drugs for kidney transplantation. *N. Engl. J. Med.* **351**, 2715–2729 (2004).
38. B. V. Naidu, B. Krishnadasan, K. Byrne, A. L. Farr, M. Rosengart, E. D. Verrier, M. S. Mulligan, Regulation of chemokine expression by cyclosporine A in alveolar macrophages exposed to hypoxia and reoxygenation. *Ann. Thor. Surg.* **74**, 899–905 (2002).
39. D. Linfert, T. Chowdhry, H. Rabb, Lymphocytes and ischemia-reperfusion injury. *Transplant. Rev.* **23**, 1–10 (2009).
40. M. Rotondi, G. S. Netti, E. Lazzeri, G. Stallone, E. Bertoni, L. Chiovato, G. Grandaliano, L. Gesualdo, M. Salvadori, F. P. Schena, P. Romagnani, M. Serio, High pretransplant serum levels of CXCL9 are associated with increased risk of acute rejection and graft failure in kidney graft recipients. *Transpl. Int.* **23**, 465–475 (2010).
41. T. Böhler, J. Nolting, N. Kamar, P. Gurrachaa, K. Reisener, P. Glander, H. H. Neumayer, K. Budde, J. Klupp, Validation of immunological biomarkers for the pharmacodynamic monitoring of immunosuppressive drugs in humans. *Ther. Drug Monit.* **29**, 77–86 (2007).
42. P. A. Corris, J. A. Kirby, A role for cytokine measurement in therapeutic monitoring of immunosuppressive drugs following lung transplantation. *Clin. Exp. Immunol.* **139**, 176–178 (2005).
43. F. H. El-Khatib, S. J. Russell, D. M. Nathan, R. G. Sutherland, E. R. Damiano, A bihormonal closed-loop artificial pancreas for type 1 diabetes. *Sci. Transl. Med.* **2**, 27ra27 (2010).
44. J. D. Andrade, V. Hlady, Protein adsorption and materials biocompatibility: A tutorial review and suggested hypotheses. *Adv. Polym. Sci.* **79**, 1–63 (1986).
45. S. Koutsopoulos, L. D. Unsworth, Y. Nagai, S. Zhang, Controlled release of functional proteins through designer self-assembling peptide nanofiber hydrogel scaffold. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 4623–4628 (2009).
46. A. S. Ahuja, W. R. Hendee, P. L. Carson, Transport phenomena in laminar flow of blood. *Phys. Med. Biol.* **23**, 928–936 (1978).

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