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Integrated Electrochemical Microsystems for Genetic Detection of Pathogens at the Point of Care

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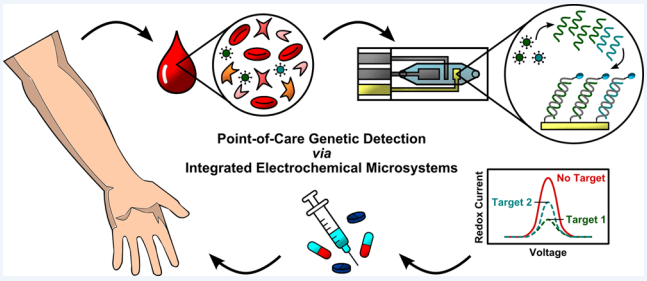
CONSPECTUS: The capacity to achieve rapid, sensitive, specific, quantitative, and multiplexed genetic detection of pathogens via a robust, portable, point-of-care platform could transform many diagnostic applications. And while contemporary technologies have yet to effectively achieve this goal, the advent of microfluidics provides a potentially viable approach to this end by enabling the integration of sophisticated multistep biochemical assays (e.g., sample preparation, genetic amplification, and quantitative detection) in a monolithic, portable device from relatively small biological samples.



Integrated electrochemical sensors offer a particularly promising solution to genetic detection because they do not require optical instrumentation and are readily compatible with both integrated circuit and microfluidic technologies. Nevertheless, the development of generalizable microfluidic electrochemical platforms that integrate sample preparation and amplification as well as quantitative and multiplexed detection remains a challenging and unsolved technical problem. Recognizing this unmet need, we have developed a series of microfluidic electrochemical DNA sensors that have progressively evolved to encompass each of these critical functionalities.

For DNA detection, our platforms employ label-free, single-step, and sequence-specific electrochemical DNA (E-DNA) sensors, in which an electrode-bound, redox-reporter-modified DNA "probe" generates a current change after undergoing a hybridization-induced conformational change. After successfully integrating E-DNA sensors into a microfluidic chip format, we subsequently incorporated on-chip genetic amplification techniques including polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) to enable genetic detection at clinically relevant target concentrations. To maximize the potential point-of-care utility of our platforms, we have further integrated sample preparation via immunomagnetic separation, which allowed the detection of influenza virus directly from throat swabs and developed strategies for the multiplexed detection of related bacterial strains from the blood of septic mice. Finally, we developed an alternative electrochemical detection platform based on real-time LAMP, which not is only capable of detecting across a broad dynamic range of target concentrations, but also greatly simplifies quantitative measurement of nucleic acids.

These efforts represent considerable progress toward the development of a true sample-in−answer-out platform for genetic detection of pathogens at the point of care. Given the many advantages of these systems, and the growing interest and innovative contributions from researchers in this field, we are optimistic that iterations of these systems will arrive in clinical settings in the foreseeable future.

■ INTRODUCTION

Genetic detection of viruses and bacteria at the "point of care" are critically needed in food safety testing,[1] in environmental monitoring,[2] and, most importantly, in clinical diagnostics.[3,4] Ideally, such detection should be sensitive, specific, quantitative, and capable of multiplexing, which necessitates multistep assays that incorporate procedures for sample preparation, genetic amplification, and quantitative detection. Point-of-care use adds still further technical challenges, as this requires that the complex, multistep procedures be performed rapidly and in a portable, robust, and user-friendly platform.[5,6] As a result, contemporary approaches have yet to achieve acceptably sensitive and specific genetic detection directly from unprocessed samples at the point of care, as evidenced by the lack of Food and Drug Administration (FDA)-approved systems.

The advent of microfluidics has lowered the technology barrier to effective, point-of-care genetic detection because it enables the development of miniaturized and disposable devices that can perform multistep biochemical assays from small sample volumes with minimal sample loss and rapid assay time

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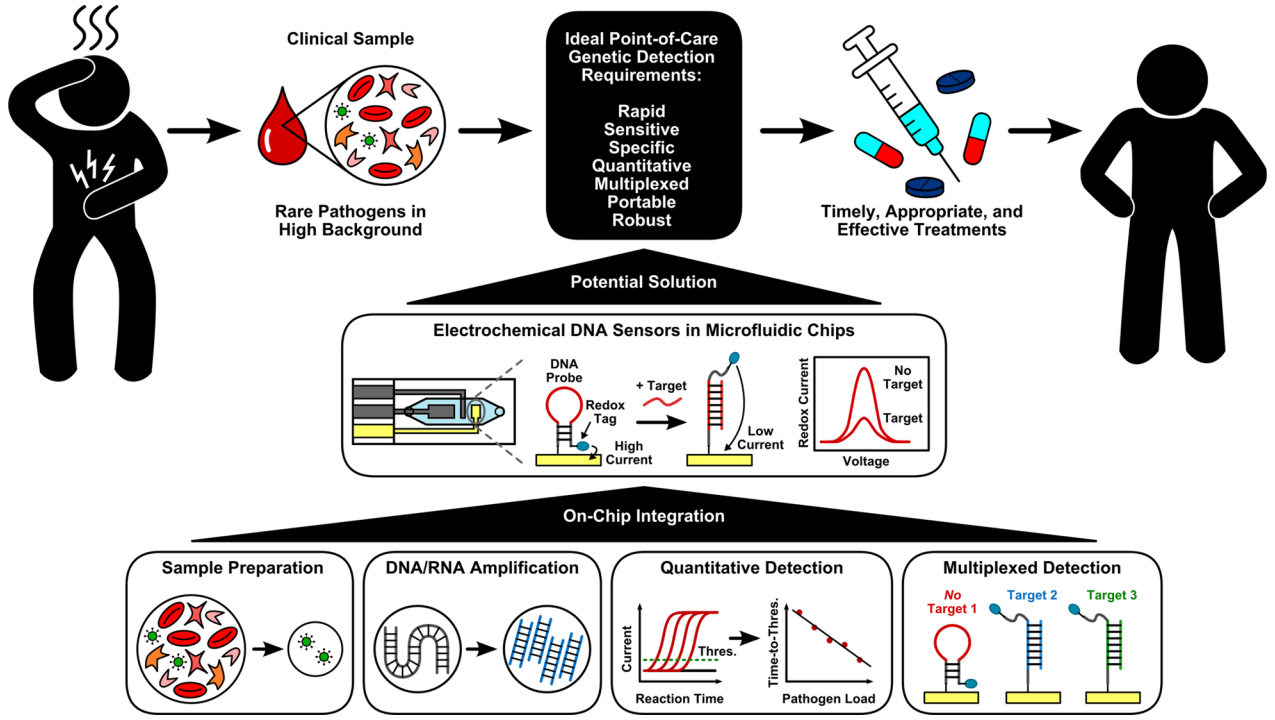

Figure 1. Evolution of integrated electrochemical microsystems for point-of-care genetic detection. Building on a foundation of microscale sensors for sequence-specific electrochemical DNA (E-DNA) target detection, we have subsequently integrated other critical on-chip functionalities, including sample preparation, DNA/RNA amplification, quantitative, real-time detection, and multiplexed detection. Integrated electrochemical microsystems that encompass these functionalities present a promising solution for genetic detection of pathogens at the point of care.

(see reviews by Chen et al.[7] and Park et al.[8]). Unfortunately, most existing examples rely on delicate and bulky optical instrumentation for detection, rendering them relatively poorly suited for point-of-care applications. Electrochemical sensors, in contrast, offer a promising alternative for simplified genetic detection as they eliminate the need for optical equipment, are highly amenable to miniaturization, and can be easily interfaced with integrated circuits and electronic instruments.[9,10] Nevertheless, the development of generalizable microfluidic electrochemical platforms that integrate sample preparation and amplification as well as quantitative and multiplexed detection remains a challenging and unsolved problem.

Motivated by the above observations, we have developed a series of microfluidic electrochemical DNA sensors that progressively incorporate integrated sample preparation, on-chip amplification, real-time measurement, and the capacity for multiplexed detection and differentiation of pathogen strains (Figure 1). In this Account, we share our perspectives on developing such microsystems toward point-of-care use, and illustrate how our developments fit in the broader context of the field by highlighting relevant contemporary systems from the literature. Finally, we briefly discuss our current research efforts and insights for future research directions.

INTEGRATION OF E-DNA PROBES IN MICROFLUIDIC CHIPS

The detection architecture we have employed in most of our platforms is the electrochemical DNA (E-DNA) sensor,[11] which employs electrode-bound DNA probes modified with a redox reporter (e.g., methylene blue, MB). Single-stranded target DNA hybridization changes the probe conformation and thereby alters the electron transfer rate of the reporter, which can be detected via a change in redox peak current (Figure 2).

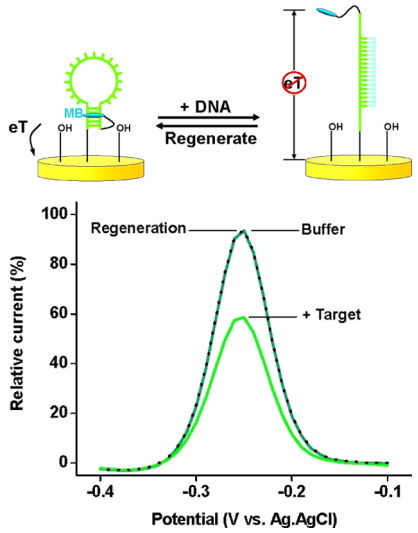

Figure 2. Operating principle of E-DNA sensors. The E-DNA sensor comprises a redox-reporter-modified DNA probe attached to an interrogating electrode. In the absence of target (left), the redox reporter is held in proximity to the electrode, ensuring efficient electron transfer (eT) and a readily detectable current. The hybridization of the target to the probe (middle) positions the redox reporter away from the electrode, thereby reducing the current signal (right). The probe can subsequently be regenerated via rinsing to restore the baseline current. Adapted from ref 13. Copyright 2006 PNAS.

Importantly, for our integrated microsystems, we have generally employed "signal-off" E-DNA sensors (i.e., target is detected by a decrease in redox peak current) because they involve simple and robust probe structures, can detect diverse DNA sequences

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(such as unpurified polymerase chain reaction (PCR) amplicons[12]) in a single step without labeling or exogenous reagents,[13] and can be efficiently regenerated via simple buffer rinsing, which serves to validate that signal change is due to the target.

A major focus of our group has been to integrate E-DNA sensors with a host of powerful microfluidic functionalities. Doing so required that we develop processes for microfluidic chip and microelectrode fabrication, electrode cleaning and preparation, electrochemical patterning and immobilization of E-DNA probes at designated sensor electrodes, sequence-specific detection from multiple electrodes, and regeneration of the sensing electrodes (Figure 3). All of these can now be performed seamlessly within a single-chambered microfluidic device.[14] Using such a device, we demonstrated sequence-specific detection of DNA target sequences derived from human (H1N1) and avian (H5N1) influenza virus (Figure 3). When challenged with H5N1 target, only the H5N1 sensor responded with a 38% signal change, while conversely, only the H1N1 sensor responded to H1N1 target with a 46% signal change. We detected these targets in a high-salt buffer at a concentration of 400 nM, which is representative of single-stranded DNA concentrations achievable via asymmetric PCR.[12] Our results were comparable to other contemporary microfluidic electrochemical DNA sensors (see reviews by Pumera et al.[15] and Mir et al.[16]).

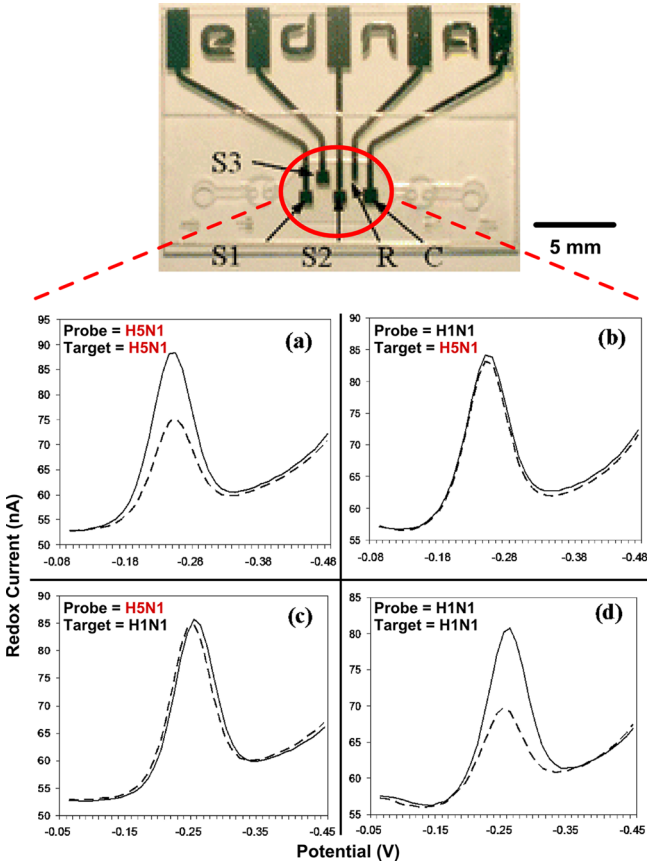

Figure 3. Specific detection of H5N1 and H1N1 in microfluidic E-DNA sensor. H5N1 target causes a current decrease for the H5N1 sensor (a) but not the H1N1 sensor (b). Conversely, H1N1 target yields negligible current change for the H5N1 sensor (c), but reduces the current at the H1N1 sensor (d). Reproduced with permission from ref 14. Copyright 2008 American Chemical Society.

INTEGRATION OF PCR WITH E-DNA

The femtomolar or even attomolar concentrations of pathogen DNA or RNA typically found in clinical samples falls below the detection limits of E-DNA and, indeed, of almost all proposed methods of (unamplified) DNA detection. Many research groups have addressed this challenge by using PCR to amplify target DNA prior to detection (see reviews by Luo and Hsing[17] and Duwensee et al.[18]), but most have kept PCR and detection as separate modules, rendering the process unsuitable for point-of-care use. In response, we developed a monolithic microfluidic system that combines PCR amplification, enzymatic conversion of PCR amplicons into single-stranded DNA, and E-DNA detection.

The Integrated Microfluidic Electrochemical DNA (IMED) sensor[19] comprises two modules: the reaction chamber and the detection chamber. Genomic DNA and PCR reagents are loaded into the reaction chamber with a syringe pump (Figure 4A), and on-chip PCR is performed using a temperature-controlled thin-film heater (Figure 4B). The reverse primers yield phosphorylated strands that are subsequently selectively digested by lambda exonuclease[20] within the reaction chamber, efficiently producing single-stranded DNA (Figure 4C and 4D). The sample and reagents are thoroughly mixed by syringe pumping in and out of the PCR chamber through a dedicated port. The single-stranded DNA is then mixed with a high-salt buffer (Figure 4E) for optimal hybridization to E-DNA probes, and pumped to the detection chamber for detection (Figure 4F).

To achieve successful operation of the IMED system required that we overcome several hurdles. For example, in order to obtain PCR efficiencies rivaling benchtop systems, we minimized the internal surface area of PDMS in our reaction chamber (known to cause enzyme adsorption), used the well-known PCR-additive bovine serum albumin (BSA) to passivate the chip surface and further reduce PCR inhibition due to adsorption, and used precise sample loading with a syringe pump to minimize air bubble formation (known to cause failure of chip-based PCR). Similarly, to ensure accurate E-DNA detection of PCR products and mitigate changes in peak current arising from the adsorption of proteins present in the PCR mix, we incubated the electrode surface with PCR mix containing BSA.

The successful integration of PCR amplification, exonuclease-driven generation of single-stranded products, and, ultimately, the detection via E-DNA into a single device allowed IMED to achieve unprecedented sensitivity. Using our chips, we have detected genomic DNA from *Salmonella enterica* serovar Typhimurium LT2 with a limit of detection (LOD) of below 10 aM (~300 copies in our 50 μL reaction chamber) (Figure 5). This is ~2 orders of magnitude lower than that of previously reported chip-based electrochemical methods,[21−24] as these platforms used less efficient, asymmetric PCR to generate single-stranded products for detection.

INTEGRATION OF SAMPLE PREPARATION

Although IMED greatly improved the sensitivity of on-chip pathogen detection, it nevertheless requires preassay isolation of pathogen DNA from samples. This increases the time required for diagnosis and creates additional work for end-users

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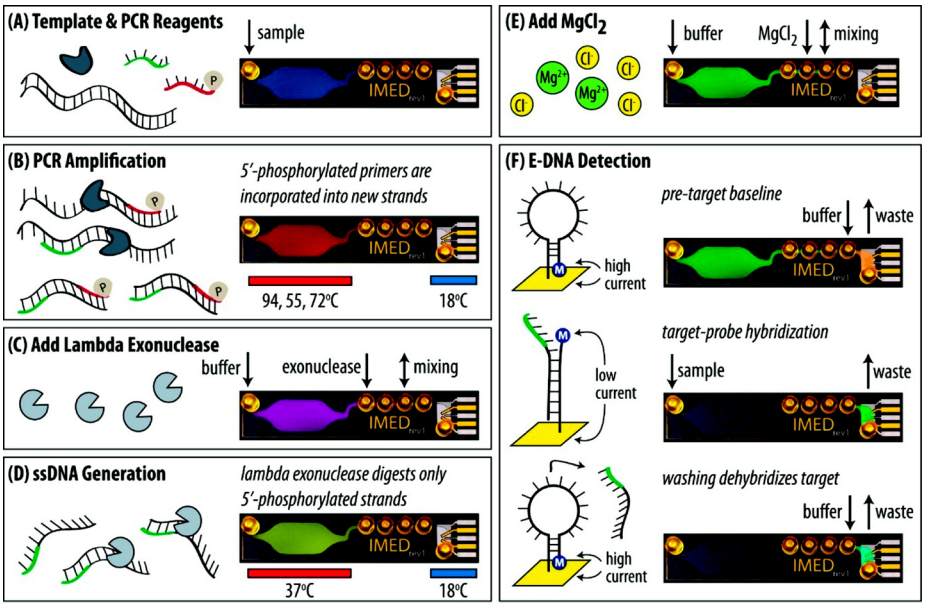

Figure 4. IMED assay overview. (A) Template DNA is added to a PCR reagent mixture containing phosphorylated reverse primers and (B) PCR amplified. (C) Lambda exonuclease is mixed with the product and (D) digests the phosphorylated strands. (E) $MgCl_2$ is added to optimize hybridization conditions. (F) Before introducing the sample, baseline sensor redox current is measured. Next, the single-stranded DNA product hybridizes with the E-DNA probe, modulating the redox current signal. Finally, the E-DNA probe is regenerated to verify target hybridization. Reprinted with permission from ref 19. Copyright 2009 American Chemical Society.

as well as opportunities for contamination or sample damage. Other existing electrochemical microsystems likewise exhibited a limited capacity for working directly with biological samples. For example, Yamanaka combined reverse-transcriptase PCR (RT-PCR) and electrochemical sensors to detect influenza A, but, as with IMED, the viral RNA was purified on the benchtop prior to on-chip amplification and detection.[25] Likewise, the microsystem presented by Safavieh et al. could rapidly detect *Escherichia coli*, but only from benchtop-purified urine samples.[26]

We therefore developed the Magnetic Integrated Microfluidic Electrochemical Detector (MIMED), which incorporates robust sample preparation functionality to overcome this bottleneck (Figure 6A).[27] The MIMED device employs a high-gradient magnetic field to enable immunomagnetic target capture, concentration, and purification (Figure 6B and C), followed by efficient on-chip RT-PCR (Figure 6D−F), single-stranded DNA generation (Figure 6G), and sequence-specific E-DNA detection (Figure 6H).

Sample preparation in the MIMED system incorporates viral RNA isolation and stabilization, magnetic-based concentration, and continuous washing, all of which are critical for the success of the assay. To isolate and stabilize intact viral RNA, we incubated the throat swab sample into a cocktail containing: (1) a nonionic detergent for dissolving the viral envelope and releasing intact ribonucleoprotein (RNP)-containing target RNA,[28] (2) an RNA stabilizer, and (3) antibody-coated magnetic beads for capturing the released RNP. We subsequently injected the sample into the device, where high magnetic field gradients captured and concentrated the bead-bound viral particles within the device, enabling us to continuously wash away cellular debris and other interferents that may inhibit RT-PCR. As such, MIMED sample preparation essentially matched the ideal, lossless positive control (viral particles doped directly into PCR mix), as measured by benchtop, real-time RT-PCR. Following this sample preparation, we used a similar workflow as in the IMED system to perform on-chip RT-PCR, lambda exonuclease-mediated single-strand generation, and sequence-specific E-DNA detection.

MIMED achieved highly sensitive H1N1 virus detection from throat swab samples within 3.5 h. Whereas virus-free negative control samples produced <1% signal change (Figure 7A), we measured sensor signals of 28%, 21%, and 4.2% from samples respectively spiked with H1N1 virus at 1000, 100, or 10 TCID50 (median tissue culture infective dose) (Figure 7B−D). This confirmed that MIMED can achieve unambiguous detection at concentrations as low as 10 TCID50, or 4 orders of magnitude lower than the clinical titers seen in typical throat swab samples (~10^5 TCID50).[29]

MULTIPLEXED DIFFERENTIATION OF TWO BACTERIA STRAINS

MIMED offers an integrated solution for directly detecting single pathogens in complex samples, but we saw even greater value in simultaneously detecting and discriminating multiple different pathogens from patient samples within a single chip. To the best of our knowledge, no other electrochemical microsystem to date has demonstrated such detection capabilities. For example, Yeung et al. achieved duplexed detection of *Escherichia coli* and *Bacillus subtilis* at concentrations equivalent to 1 × 10^5 cells/mL, but only from culture broth.[23]

To achieve this, we developed an integrated device that performs loop-mediated isothermal amplification (LAMP)[30] to "universally" amplify common gene regions of closely related *Salmonella* strains directly from blood samples and subsequently achieves detection and strain discrimination with two sequence-specific E-DNA probes functionalized on two distinct electrodes (Figure 8).[31] As a demonstration, we designed our assay to detect *S. enterica* subsp. enterica serovars Typhimurium and Choleraesuis (causative agents of enter-

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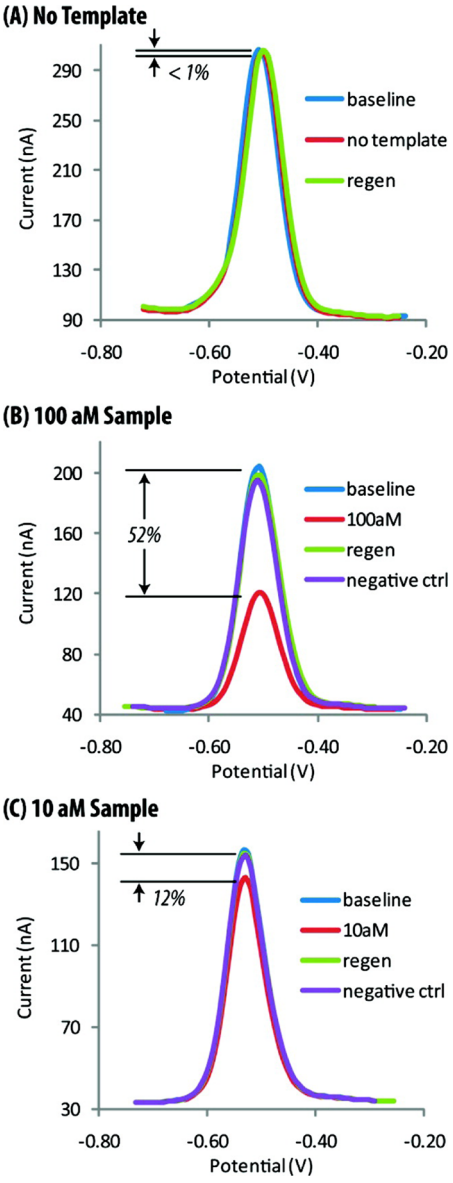

Figure 5. IMED detection of Salmonella genomic DNA. (A) The no-template negative control yielded <1% change in current (red) compared to the baseline (blue). Probe regeneration reset the sensor to within 98% of its initial state (green). The (B) 100 aM and (C) 10 aM samples produced a 52% and 12% signal change, respectively, relative to the baseline. Each measurement was validated via sensor regeneration (green), as well as benchtop-prepared zero-template negative controls, which resulted in drops of 1% and 0%, respectively (purple). Reprinted with permission from ref 19. Copyright 2009 American Chemical Society.

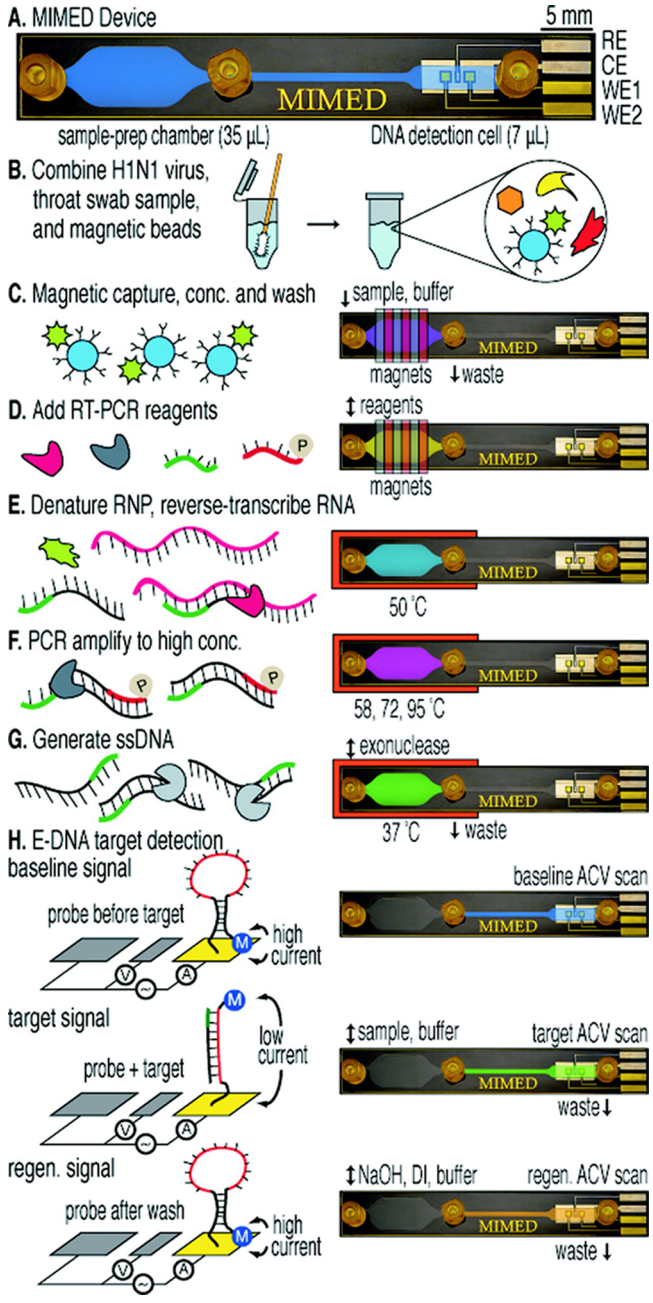

Figure 6. MIMED achieves sample-to-answer genetic detection of H1N1 virus from throat swabs. (A) The device features a sample preparation/reaction chamber, an E-DNA detection chamber, and three fluidic ports: sample/buffer/reagent input (left), waste output (center), and E-DNA product output (right). (B) A throat swab is collected and combined with influenza virus and antibody-coated magnetic beads in a tube containing RNA stabilizer. (C) The sample is pumped into the sample preparation chamber, where external magnets capture, concentrate and purify labeled viral RNPs. (D) RT-PCR mix is injected, and (E) The chip is heated to denature the RNP and release the RNA. (F, G) RT-PCR is performed on-chip, followed by lambda exonuclease-mediated single-strand generation. (H) The product is then pumped into the detection chamber for detection. Reprinted with permission from ref 27. Copyright 2011 American Chemical Society.

ocolitis and sepsis in humans[32]) directly from unprocessed, whole blood of infected mice.

We employed LAMP rather than PCR as the amplification technique in this work due to its sensitivity, robustness, isothermal reaction condition, and most importantly, the rapid assay turnaround time, which is crucial for point-of-care diagnostics. Specifically, our LAMP was sufficiently robust to work in up to 10% blood by volume with essentially no sample preparation and produced a high concentration of single-stranded amplicons that can be detected with E-DNA without the need for an additional step to generate single-stranded products. Coupled with the inherently fast reaction speed of LAMP (~1 h), this considerably shortened the assay turnaround time from 3.5 h (in the case of MIMED) to <2 h.

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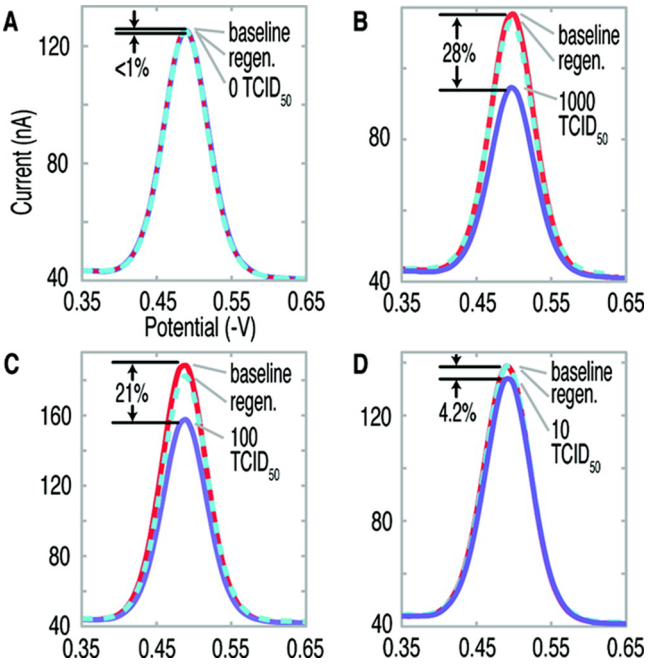

Figure 7. Sensitive, sample-to-answer detection of H1N1 from throat swabs with MIMED. Relative to the 0.5% peak current change observed for (A) our virus-free negative control, swab samples containing (B) 1000, (C) 100, and (D) 10 TCID50 return clearly detectable peak current changes of 28, 21, and 4.2%, respectively. All sensors could be regenerated to baseline levels, verifying the presence of the target in the sample. Reprinted with permission from ref 27. Copyright 2011 American Chemical Society.

After the LAMP reaction, detection of strain-specific internal amplicon sequences was achieved with two sequence-specific E-DNA probes functionalized on two distinct electrodes using an established "differential probe labeling" technique.[14] In this, we first immobilized *S.* Typhimurium specific probe onto both electrodes, then selectively desorbed the probe molecules from the second electrode by applying a positive potential sweep, and finally immobilized the exposed second electrode with *S.* Choleraesuis specific probe.

The resultant device readily differentiated the two strains in unprocessed blood from septic mice at clinically relevant levels of <1000 colony-forming units (CFU)/mL (Figure 9). Animals

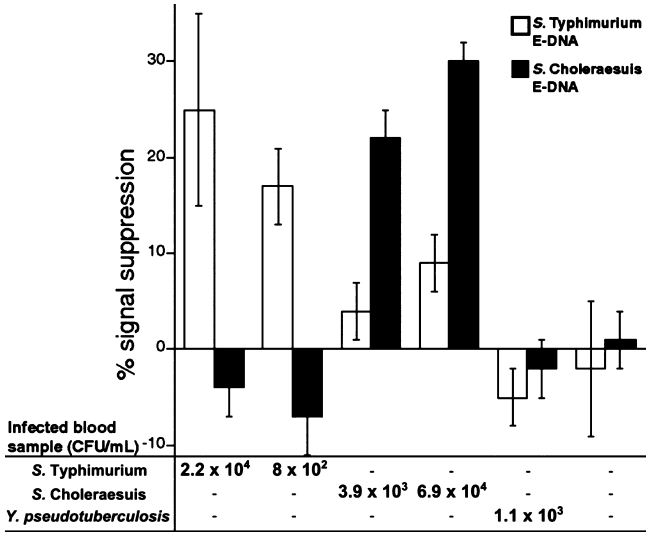

Figure 9. LAMP chip-based detection and discrimination of Salmonella serovars. Our LAMP chip could detect and differentiate *S.* Typhimurium from *S.* Choleraesuis from blood samples derived from septic mice infected with these bacteria. In contrast, samples from uninfected or *Y. pseudotuberculosis* infected mice yielded minimal signal change. CFU/mL for each sample was measured by direct colony counting. Reproduced from ref 31. Copyright 2013 American Society for Microbiology.

were infected intraperitoneally with 1000 CFU of *S.* Typhimurium, *S.* Choleraesuis or *Yersinia pseudotuberculosis*, with uninfected mice as negative controls. We added blood collected from the tail vein at day 5 postinfection to the LAMP reaction mixture and loaded it into the LAMP chip, with an additional aliquot of blood reserved to quantify CFUs by direct

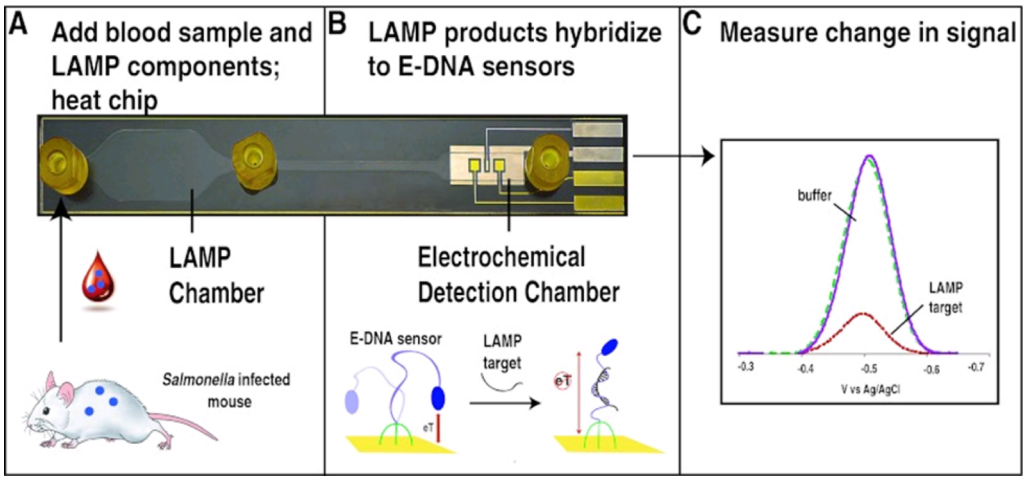

Figure 8. Overview of the LAMP chip assay. (A) Unprocessed, whole blood from infected animals is introduced into the chip's amplification chamber along with LAMP reagents and heated at 65 °C. The reaction mixture containing single-stranded amplicons is then pushed into (B) the electrochemical detection chamber. This chamber contains a duplexed electrode array that supports simultaneous, sequence-specific electrochemical detection by selectively hybridizing with amplicons from *S.* Typhimurium or *S.* Choleraesuis, (C) generating a detectable decrease in current. Reprinted from ref 31. Copyright 2013 American Society for Microbiology.

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plating. Our LAMP chip could discriminate *S.* Typhimurium from *S.* Choleraesuis in blood containing between 8×10^2 and 6.9×10^4 CFU/mL. In contrast, samples from uninfected or *Y. pseudotuberculosis*-infected mice (at 1.1×10^3 CFU/mL bacterial load) yielded minimal signal change, validating our assay.

REAL-TIME ELECTROCHEMICAL DETECTION

The detection systems described above could yield a valuable yes/no answer or semiquantitative results, but do not possess the fully quantitative capabilities that would be desirable in a clinical diagnostics setting. This is because the final concentration of amplification products generally does not correlate well with the initial copy number present in a sample. In response, we have developed the microfluidic electrochemical quantitative (MEQ)-LAMP platform,[33] which allows for continuous electrochemical monitoring of LAMP reaction progress in real-time, facilitating quantitative measurements. MEQ-LAMP leverages the fact that the MB redox reporter can intercalate into double-stranded DNA to directly detect production of double-stranded LAMP amplicons. The MEQ-LAMP chip features a single chamber for both amplification and detection (Figure 10A). Initially, MB molecules doped into

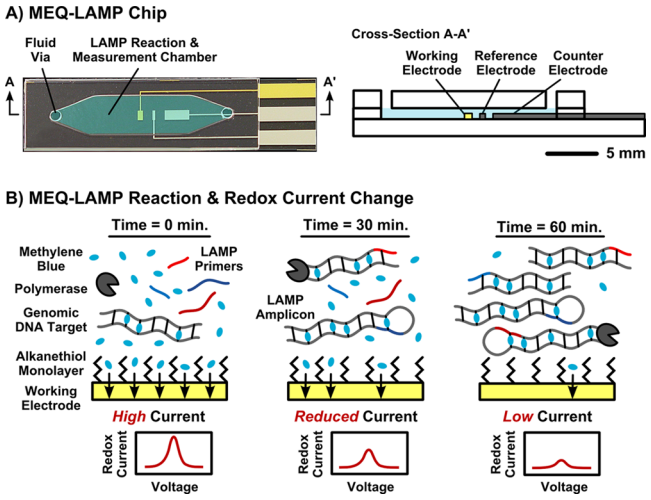

Figure 10. Overview of MEQ-LAMP. (A) The MEQ-LAMP chip features a single chamber for both amplification and electrochemical detection. (B) MB enables real-time monitoring of the LAMP reaction. Initially, MB molecules doped into the reaction mix freely encounter the gold working electrode and transfer electrons, producing a measurable current. As the reaction progresses, intercalation of MB molecules into the LAMP amplicons segregates them from the electrode, proportionally decreasing the current. Reprinted with permission from ref 33. Copyright 2012 Wiley.

the LAMP reaction mix freely encounter the gold working electrode and transfer electrons, producing a measurable current. As the reaction progresses, MB intercalates into the double-stranded amplicons. This segregates MB from the electrode, decreasing the redox current in a manner that enables tracking of the reaction in real time (Figure 10B).

MEQ-LAMP is considerably simpler than our previous platforms; this detection mechanism completely obviates the need for E-DNA probes or probe-target hybridization, though it requires a new strategy for data processing. Once genomic DNA and the MB-doped LAMP reaction mixture are loaded, the chip is mounted onto a block heater maintained at 65 °C. As the reaction proceeds, we initiate a series of time-course voltammetry scans, measuring the current every minute throughout the reaction. We generally observed an initial decrease in the redox current traces in the first 10 min for all of our reactions, regardless of the presence of DNA target, but only LAMP reactions containing the target of interest triggered a further decrease, yielding a sigmoid pattern resembling the reaction kinetics typically observed in real-time PCR and LAMP (Figure 11A). This sigmoidal curve enabled us to define

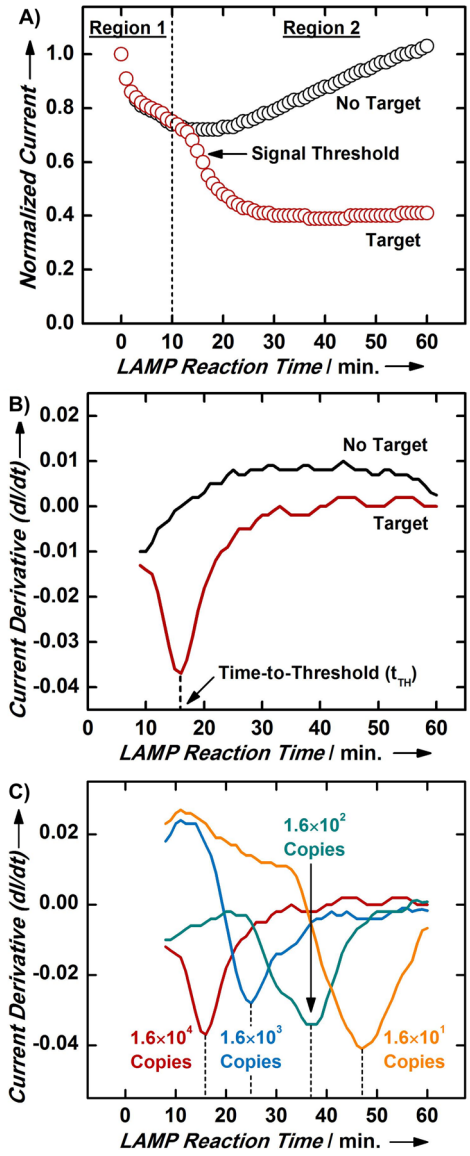

Figure 11. MEQ-LAMP accurately quantifies genomic DNA target copy number. (A) Normalized real-time current traces for a negative control sample (black) and a sample containing *S.* Typhimurium genomic DNA (red). Only the pathogen-containing sample generates a sharp current decrease in Region 2 of the current trace, with the sigmoidal behavior similar to typical real-time PCR kinetics. (B) By taking the derivative of the current trace and defining the signal threshold as the local minimum in the derivative curve, we can determine the required reaction time to threshold (t_{TH}, vertical dashed line). (C) 10-fold serial dilutions of *S.* Typhimurium DNA (ranging from 1.6×10^4 to 1.6×10^1 copies) result in traces with distinct t_{TH}, separated by approximately 10 min. Reprinted with permission from ref 33. Copyright 2012 Wiley.

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the signal threshold for each MEQ-LAMP reaction at the local minimum in the corresponding current derivative trace (i.e., dI/dt). We defined the time to threshold (t_{TH}) as the time required for a particular sample reaction to reach the signal threshold (Figure 11B), analogous to the threshold cycle (C_T) concept in real-time PCR.

Using this strategy, MEQ-LAMP measures Salmonella genomic DNA with a dynamic range spanning 3 orders of magnitude and a LOD as low as 16 copies in <50 min. Different initial copy numbers of *S.* Typhimurium DNA (10-fold serial dilutions ranging from 1.6×10^4 to 1.6×10^1 copies) generated temporally distinct local minima separated by approximately 10 min for each of the current derivative traces, with a distinct t_{TH} for each initial target copy number (Figure 11C). MEQ-LAMP's speed, sensitivity, and quantitative capabilities surpassed other real-time electrochemical amplification platforms (see review by Patterson et al.[34]), thus triggering growing interests in this approach. Other research groups have subsequently developed platforms employing similar strategies to achieve impressive performance.[35−38] For example, Ahmed et al.[36] have reported detection of *Staphylococcus aureus* and *Escherichia coli* at LODs of 30 and 20 copies μL^{-1}, respectively, in 30 min, and Luo et al.[37] and Safavieh et al.[38] have both demonstrated real-time, multiplexed on-chip electrochemical LAMP.

■ CONCLUSIONS AND OUTLOOK

In this Account, we have described our evolving efforts in developing integrated electrochemical microsystems for the genetic detection of pathogens at the point of care. Building on a foundation of microscale sensors employing redox-tagged, conformation-switching E-DNA probes realized in microfluidic chips, we have subsequently incorporated on-chip genetic amplification to enable detection at clinically relevant concentrations. The next generation of devices further simplified clinical application by integrating sample preparation via immunomagnetic separation or even direct amplification in blood via LAMP. We have further demonstrated the potential for multiplexed pathogen differentiation by discriminating two bacterial strains in blood. Most recently, we have achieved rapid, quantitative, real-time detection across a broad dynamic range of nucleic acid concentrations via the MEQ-LAMP platform. This approach has now become popular in this field, and we foresee exciting potential to incorporate sample preparation and multiplexed detection capabilities into the MEQ-LAMP platform.

Though the design of an integrated electrochemical microsystem must be dictated by the specific application—the pathogen or pathogens of interest, the type of biological sample, and the type of sample preparation (see specific applications provided by Park et al.[5] and Niemz et al.[6])—we see several general areas to further extend the utility of integrated electrochemical microsystems and accelerate their implementation at the point of care. Effective sample preparation remains a critical challenge.[6,39,40] Direct target amplification in biological samples offers one simple solution without adding to the assay turnaround time, but still-greater sensitivity is required to detect the limiting quantities of pathogen genetic material in microliter-scale sample volumes. Immunomagnetic purification and concentration are effective, but require additional reagents and steps that may prove problematic in point-of-care settings. Furthermore, alternative approaches are needed for pathogens that are difficult to lyse (e.g., Gram-positive bacteria) and biological samples that require specialized processing (e.g., sputum).

Expanded multiplexing would be far more efficient than single-pathogen assays, and could facilitate treatment by enabling discrimination of distinct pathogens that cause similar disease states (e.g., sepsis). By some estimates, genetic testing for ∼8−30 different microbial strains could diagnose 80−90% of all serious infections, facilitating proper antibiotic selection.[41] Performing multiple pathogen-specific reactions in parallel is one solution, but this approach can be inefficient and cost-prohibitive for large numbers of targets. Alternatively, one might employ universal primers to amplify sequences that can be subsequently differentiated via either sequence-specific probes (as demonstrated with our LAMP chip and elsewhere[42−45]) or labeled with different redox reporters.[46,47] However, the extent to which this approach can be scaled up remains to be seen.

System integration, automation, and miniaturization will also play a critical role.[48] Current microsystems generally still require manual intervention (e.g., reagent loading) and relatively bulky peripheral instruments (e.g., syringe pumps, heating blocks, and potentiostats). Potential solutions to this challenge may be integrated platforms that support multistep assays and electrochemical detection in a hands-free fashion, such as centrifugal microfluidic devices,[49] bubble-mediated reagent transfer devices,[50] and paper microfluidic devices.[51] We note that reagents must also be embedded in such devices to ensure true point-of-care use. In parallel, portable instruments such as commercially available, miniature, USB-powered potentiostats and custom-developed potentiostats[52,53] have grown increasingly popular as means to reduce system footprint.

In sum, the past several years have witnessed significant advances in integrated electrochemical microsystems, and the technological barriers that once thwarted the development of these systems have been lowered considerably. Given their many advantages, the promising recent advances described above, and the growing number of research groups making valuable contributions to this field, we are optimistic that future iterations of integrated electrochemical microsystems will address the remaining challenges in sample preparation, multiplexed detection, and system integration, and thereby bring the power of genetic detection of pathogens to the point of care in the foreseeable future.

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