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Chemistry-Driven Approaches for Ultrasensitive Nucleic Acid Detection

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ABSTRACT: Methods that can rapidly and specifically analyze nucleic acid sequences will revolutionize the diagnosis and treatment of disease by allowing molecular-level information to be used during routine medicine. In this Perspective, we discuss chemistry-driven approaches that will make the detection of DNA and RNA sequences more routine in clinical settings. In addition, we discuss unmet needs and areas where future effort is necessary to enable nucleic acids analysis to become a mainstream tool in routine clinical medicine. Methods for next-generation sequencing of DNA are producing a wealth of information by allowing the study of how specific genetic mutations or single nucleotide polymorphisms influence the onset of disease, prognosis, or response to treatment. To give this information clinical utility, new methods of detecting nucleic acid sequences are being developed in order to rapidly obtain genetic information in more streamlined formats, and with the ability to obtain information outside of a laboratory setting. Challenges remain in this area, however, and new chemistries that will facilitate fast, simple nucleic acids analysis in a clinical setting are needed.

1. INTRODUCTION

The analysis of nucleic acids sequences is a critical capability for the routine implementation of personalized medicine and effective diagnosis and management of cancer and infectious disease. Genotypic analysis has already proven to be crucial in a variety of applications. Antibiotic resistance can be conferred to *Staphylococcus aureus* via acquisition of the *mecA* gene, and the most effective methods for the identification of methicillin-resistant *S. aureus* (MRSA) detect the presence of this DNA sequence.[1] Hepatitis C viral (HCV) infections possess one of at least six genotypes, and different types are treated with different therapeutic regimens.[2,3] Genotypic analysis is therefore critical for effective management of this infection. Breast cancer tumors are routinely tested for the gene encoding human epidermal growth factor receptor 2 (HER2),[4] which is overexpressed in more aggressive cancers and necessitates treatment with drugs that specifically target HER2.[5] The analysis of gene expression also has significant utility in transplantation medicine, as analysis of mRNA levels of cytokine expression can help predict survival rates for lung transplants.[6] As summarized in Figure 1, these are just a few examples of the roles that nucleic acid analysis can play in the management of disease.

Many of the applications of nucleic acid analysis listed above were based on discoveries that occurred early in the genomics-focused era. Over the past decade, developments in next-generation sequencing techniques have spurred the generation and analysis of large amounts of sequencing data, and permitted the decoding of entire genomes. Obtaining sequencing data encompassing the entire human genome at a cost approaching 1000 USD is now possible.[7] Analyzing and understanding this genomic data will lead to discoveries uncovering how genomic differences manifest in disease development and progression in individuals.[8] With these technological advancements, whole genome sequencing is becoming a clinical tool, leading a move toward personalized medicine based on an individual's genetics. However, for many applications that require rapid results, new analytical methods will be needed to make the routine testing for DNA and RNA biomarkers cost-effective and practical for mainstream medicine.

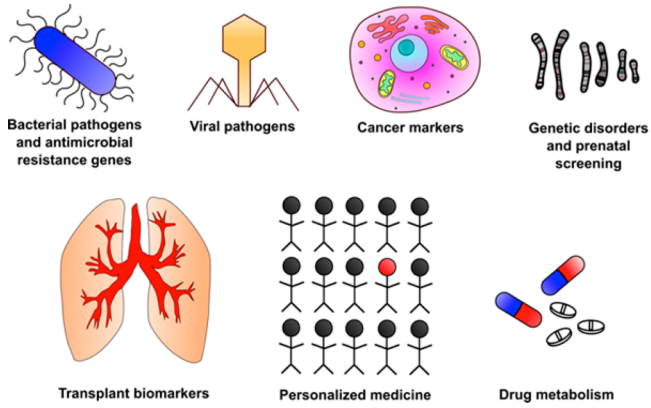

Figure 1. Targets and applications for nucleic acid detection assays. Examples of detection targets and applications where fast, cost-effective nucleic acid analysis could revolutionize medical diagnostics and treatments.

The polymerase chain reaction (PCR)[9,10] and other enzymatic amplification approaches are workhorses in clinical diagnostic laboratories. However, the very high levels of sensitivity that can be obtained with PCR makes this technique susceptible to false positives produced by contamination[11] and trained personnel and sophisticated lab facilities are required for sample processing. Furthermore, the multiplexing of this approach can be limited by the number of available instrumentation channels to detect different fluorescent probes for different targets.[12] The development of new modalities for multiplexed molecular-level testing that can be deployed at the bedside or in resource-limited settings to quickly aid in disease

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Table 1. Summary of Selected Nanomaterial-Based Nucleic Acids Detection Approaches

material	representative limit of detection	detection method	turnaround time (including sample preparation)	requirements for sample preparation/sample input	key advantages
metal nanoparticles	50 fM[18]	optical	>1 day	purified nucleic acids	sharp melting transitions; discrimination of SNPs
DNA nanostructures	10 aM[36]	electrochemical	<1 day	purified nucleic acids	enhanced probe display
carbon nanotubes	5 aM[43]	electrochemical	<4 h	purified nucleic acids	can be interfaced with a variety of electrode materials
silicon nanowires	0.1 fM[47]	electrical	<1 h	purified nucleic acids	one of the few nanomaterials that can be individually addressed
nanostructured metal electrodes	10 aM (oligo)[55] 1 fM (mRNA)[57]	electrochemical	<1 h	compatible with crude cellular lysates	rapid readout

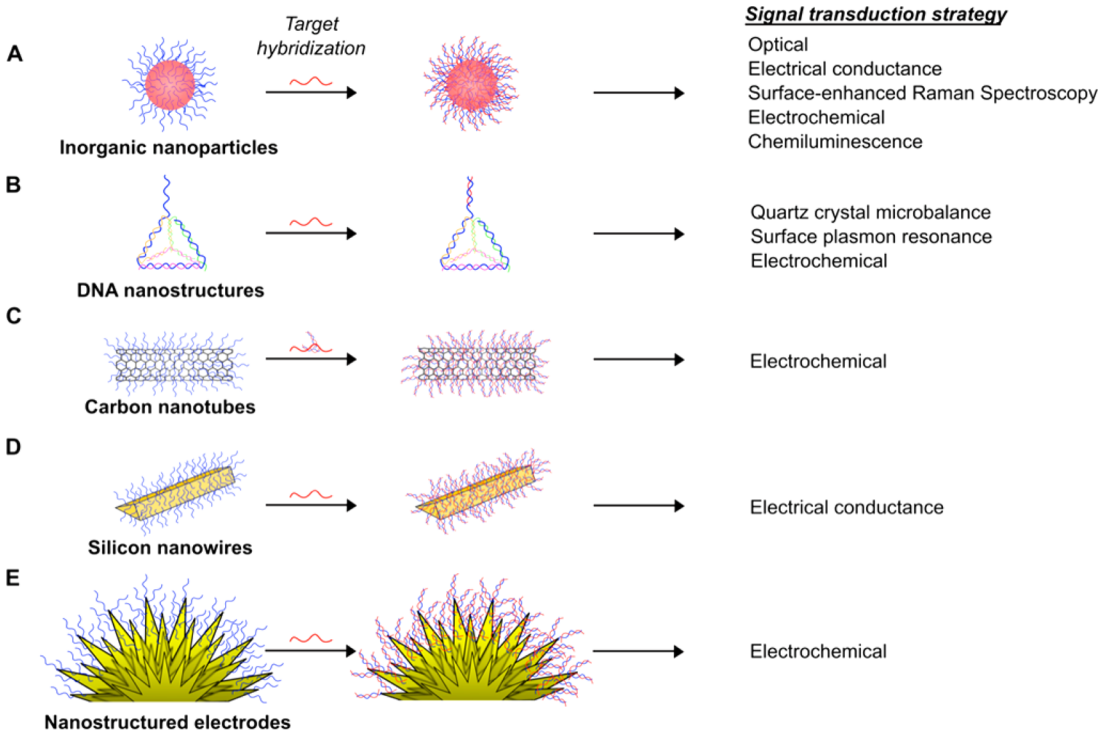

Figure 2. Nanomaterial-based approaches for rapid nucleic acid analysis.

diagnosis or genotyping is essential to fully utilize the wealth of genomic information increasingly available concerning the molecular basis of disease.

Detecting specific nucleic acid sequences in clinical specimens is a formidable challenge. Very high levels of sensitivity coupled with stringent specificity are required to sort through the heterogeneous collections of sequences found in real-life samples. PCR circumvents this problem by copying a sequence of interest until it outnumbers all of the background material. But can chemical methods enable direct detection without the need for this type of physical amplification of target material? This is an important goal to work toward, as direct detection may present a means to overcome the limitations of PCR and provide a breakthrough that would allow DNA and RNA analysis to become routine.

In order to move away from methods of nucleic acid detection that rely on PCR or other types of enzymatic amplification, new direct detection approaches must exhibit high levels of sensitivity and specificity. Sensitivity levels must be compatible with the low levels of nucleic acids in clinical samples (attomolar to picomolar) to avoid false negatives, and high levels of specificity are required to minimize false positives.

Finding ways to automate detection assays so that they can be moved out of research and testing labs and toward the clinic is also critical. Since clinical testing for biomolecular analytes must be foolproof, straightforward and standardized methods for conducting assays must be devised. Devices that systematically move fluids using microfluidics or other approaches provide a promising path for the translation of nucleic acid detection approaches for clinical applications.

In this Perspective, we discuss new chemistry-driven tools that are being developed for nucleic acid analysis. This is a very large, fast-moving field, and therefore we focus on three areas that we believe will make a significant, near-term impact on the development of high-sensitivity methods that are versatile and scalable. We discuss the use of nanomaterials as biomolecular detectors, which has prompted significant improvements to detection limits that can be obtained with nucleic acid detection assays; new signal transduction mechanisms with built-in amplification that will provide alternatives to PCR; and microfluidic approaches to the automation of nucleic acid detection assays. In reviewing progress in these areas, we offer perspective on why these advances should provide new

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streamlined solutions for providing rapid genotypic information and allow for routine personalized medicine.

2. NANOMATERIALS ENHANCE THE DETECTION OF DNA AND RNA AT THE NANOSCALE

Nanomaterials possess a variety of advantages as biomolecular detectors. Large surface-to-volume ratios coupled with nanoscale dimensions allows target binding events to lead to large signal changes. Moreover, nanomaterials often possess versatile structures and tunable physical properties that can be honed to a particular application. As well, the display of probe molecules on surfaces with molecular-scale curvature can enhance target binding and sensitivity. For all of these reasons, nanomaterial-enabled assays for DNA and RNA continue to be popular and are breaking performance records related to the speed and sensitivity of nucleic acids detection (Table 1). The use of nanomaterials has allowed performance levels achieved in direct detection assays to compete with enzymatic approaches like PCR, with aM concentrations being detectable with many approaches. We summarize several promising systems below and comment on remaining challenges.

Inorganic Nanoparticles. One of the first nanomaterial-based approaches that exhibited performance levels matching the requirements of clinical testing used spherical nucleic acids (SNAs) containing gold nanoparticle cores functionalized with thiolated single-stranded DNA (Figure 2A).[13] Gold is one of the most popular materials for molecular functionalization given the spontaneous adsorption of thiols to gold that allows for straightforward surface modification.[14] SNAs can be engineered to aggregate in the presence of specific nucleic acid sequences, and a resultant color change can be used as a readout strategy. Particle aggregation causes a surface plasmon resonance shift for gold nanoparticles, generating color shifts in solution from red to purple.[13,15] A similar phenomenon was later reported for unmodified gold particles that were stable in the presence of single-stranded DNA, and that aggregated in the presence of double-stranded DNA due to changes in electrostatic properties.[15]

One very interesting and useful property of SNAs is the ability to impart narrower melting temperature (T_m) ranges to double-stranded DNA compared to solution-borne duplexes.[16] This effect was exploited for sequence-specific detection of oligonucleotides, and allowing the differentiation down to one base pair mismatch, insertion or deletion.[17]

Although SNAs display high sequence selectivity, further amplification is needed to achieve femtomolar sensitivity levels. One strategy used to boost sensitivity exploited the catalytic properties of gold nanoparticles in three-component sandwich assays.[18] Surface-functionalized oligonucleotides were used to capture target DNA that was also complementary to SNAs. The gold cores of the anchored SNAs then reduced silver ions, forming a black coating on gold that was visible by eye or by using a flatbed scanner. The silver enhancement lowered detection limits and single base pair mismatches were detectable through T_m analysis.

Other approaches for DNA detection using metal nanoparticles include assays where gold or silver nanoparticles are captured in sandwich assays and oxidatively dissolved into their composite ions. Electrochemical[19,20] detection or chemiluminescent reaction systems based on luminol can be used for catalytic amplification.[21,22] A similar nanoparticle dissolution approach was employed with ZnS, CdS and PbS quantum dots (QDs) for multiplexed electrochemical DNA detection.[23] QDs have been used widely in other types of DNA and RNA detection assays.[23−29]

Nanoparticle-based DNA and RNA detection approaches have achieved impressive levels of sensitivity, but typically require adaptation into an array-based format for practical use. Looking at large ensembles of nanoparticles is needed so that the optical changes upon target binding can be detected. Performance levels may be enhanced further as readout strategies are developed with adequate sensitivity for the analysis of single nanoparticle complexes.

DNA Nanostructures. DNA sequences immobilized on gold surfaces have been used widely to create sensors for nucleic acid detection. However, short DNA sequences lacking secondary structure may nonspecifically adsorb to surfaces, limiting the efficiency of target strand binding. A need therefore exists for alternative DNA structures to optimize sensitivity for use in diagnostics.[30,31] Three-dimensional (3D) DNA tetrahedron-shaped nanostructures are a new type of scaffold for controlled probe density and orientation on surfaces, eliminating strand entanglement (Figure 2B).[32,33] Tetrahedron-structured probes (TSPs) self-assemble from four single-stranded DNA sequences.[34] TSPs can be oriented on gold surfaces through thiol groups at three vertices. The fourth vertex contains an exposed pendant single-strand DNA probe for hybridization.

The use of biotin-tagged reporter strands that bind the target nucleic acid sequence allowed for the introduction of avidin-horseradish peroxidase (HRP) complexes as a reporter strategy.[34] This complex facilitates electrochemical DNA detection via the enzymatic breakdown of H_2O_2. Though sensitivity was only 1 pM, the method could detect SNPs and was also compatible with detection of DNA in serum, showing minimal protein adsorption to the surface. The sensitivity of this platform was further improved by increasing the catalytic activity in the system.[35,36]

DNA nanostructures are an intriguing element for the development of high-performance sensors given their demonstrated ability to enhance the activities of probe sequences and promote target binding. Further application of these structures may be impeded, however, because of the vulnerability of DNA to naturally occurring nucleases. This vulnerability could be countered with the use of artificial nucleic acids, which are less characterized as elements in DNA nanostructures but should be able to recapitulate the properties of the structural templates already developed.

Carbon Nanotubes. Carbon nanotubes have been exploited as nanoelectrodes for electrochemical detection of DNA due to their ability to accumulate nucleic acids (Figure 2C).[37,38] Multiwall carbon nanotubes (MWCNTs) and single-wall carbon nanotubes (SWCNTs) have been shown to enable adsorptive accumulation of adenine and/or guanine, amplifying their electrochemical oxidation signal.[38,39]

Carbon nanotubes are typically used as ensembles in biomolecular detection assays, and are well-suited for integration with other electrode materials for sensor fabrication. By coating nanotubes on glassy carbon electrodes[38] and paste electrodes,[40] DNA sequences such as a segment of the *BRCA1* gene were detected.[38] Direct DNA functionalization of MWCNT arrays embedded vertically in silicon oxide has also been exploited and paired with $Ru(bpy)_3$ as a guanine redox mediator for electrochemical amplification, resulting in detection limits lower than a few attomoles of oligonucleotides.[41] Furthermore, MWCNTs have been functionalized with

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Table 2. Summary of Signal Amplification Approaches

signal amplification approach	representative limit of detection	detection method	turnaround time (including sample preparation)	requirements for sample preparation/sample input	key advantages
detection of DNA via personal glucose monitors	40 pM[64]	electrochemical detection of glucose as signal transducer	<1 day	fragmented DNA	uses commercially available, cost-effective instrumentation
single-molecule array (Simoa) DNA assay	70 aM[65]	fluorescence	<12 h	denatured and fragmented DNA	enables precise quantitation
synthetic gene circuits	1 fM[75]	fluorescence/visual	<4 h	heat-based lysis	easily reprogrammable to detect emerging targets

alkaline phosphatase (ALP) for electrochemical signal amplification and streptavidin to serve as a recognition element to detect DNA captured in a sandwich format.[42] Detection limits in this system were lowered from fM[42] to aM concentrations by increasing enzyme density per nanotube through layer-by-layer deposition.[43]

Silicon Nanowires. Semiconductor silicon nanowire (SiNW) field effect transistors have been used for the sensitive quantification of nucleic acids. Both p- and n-type SiNWs have been functionalized with neutrally charged peptide nucleic acids (PNAs) to detect concentration-dependent changes in conductance upon negatively charged DNA hybridization (Figure 2D).[44−46] Using p- and n-type PNA-SiNWs independently, 10 fM detection limits were reported and one- or two-base-pair mismatches as well as codon deletions were detectable in sequences such as miRNA extracted from Hela cells and the cystic fibrosis transmembrane receptor gene, respectively. By optimizing parameters such as gate voltage, buffer ionic strength and probe concentration further, a detection limit of 0.1 fM was achieved.[47] Arrays combining p- and n-type SiNWs have also been developed for nucleic acid detection, reducing false-positive signal production and electrical cross-talk.[48] Furthermore, the array format, containing 16 aligned SiNWs, allowed for multiplex detection of two influenza DNA sequences down to 1 fM concentrations and with SNP discrimination.[49]

SiNWs are one of the few nanomaterials that have been addressed as individual nanostructures in biomolecular detection arrays. This is a potential advantage as it should allow the analysis of very small collections of molecules, but single nanowire measurements are also inherently difficult and prone to noise created by environmental conditions. Robust methods for the reliable fabrication of SiNW arrays are needed to make this type of approach scalable for the production of practical devices.

Nanostructured Electrodes as High Sensitivity Detectors. Our laboratory has worked extensively with nanostructured electrodes as a platform for ultrasensitive nucleic acids detection (Figure 2E). In studies where thiolated DNA[50,51] or PNA[52] capture probes were attached to gold nanowires and an electrocatalytic reporter system was used to detect hybridization, a significant enhancement in detection sensitivity was observed relative to studies conducted with conventional electrode materials. One source of the enhancement observed related to the more favorable diffusion profile of the nanowires: nanowires enhanced the redox cycling underlying electrocatalysis, as they allowed for radial diffusion around the electrodes, compared to two-dimensional platforms that are limited to linear diffusion.[50]

To reproduce the enhanced performance observed with nanowire electrodes in a more scalable system, chip-based nanostructured microelectrodes (NMEs) were developed.[53−57] The structures were grown through electrodeposition on silicon wafers patterned with circular apertures via conventional photolithography techniques and were functionalized with thiolated PNA probes. The nanostructuring of the electrodes was adjusted by tuning electrodeposition parameters such as plating potential and deposition time.[55] Finer and more highly branched nanostructures displayed lower detection limits (10 aM) compared to moderately structured and smoother electrodes with 10 fM and 100 fM detection limits, respectively. It was proposed that the increased texturing improved the activity of probes displayed on the finer nanostructures.

To achieve the detection of clinically relevant analytes like mRNAs, large surface area NMEs were developed to overcome the slow diffusion of large molecules. With a 100 μm footprint, it was shown that bacterial RNA could be detected on chip in 20 min.[54,56] These sensors have also been adapted to analyze antibiotic resistance markers,[53,58,59] cancer biomarkers,[58,59] and sequences associated with lung transplant outcomes.[60]

Nanostructured electrodes are another example of a nanomaterial analyzed as an ensemble to increase the level of signal generated upon analyte capture to detectable levels, and to maximize capture of large, slow-moving targets via large footprint sensors. Other reports have utilized metal nanoparticles deployed in solution to first capture the analyte; these particles are then collected for electrochemical analysis in model systems.[61] This method may represent a means to make nanostructure-mediated electrochemical detection even more powerful.

3. NEW SIGNAL AMPLIFICATION APPROACHES FOR DIRECT DETECTION OF NUCLEIC ACIDS

The development of new signal amplification approaches is another important area where new breakthroughs are needed for advanced biomolecular sensing systems to exhibit clinically relevant levels of performance. Several new systems that achieve impressive levels of sensitivity and precision have been reported that present new options for biomolecular detection assays. The key features of these approaches are summarized below and in Table 2.

Utilization of Enzymatic Signal Amplification and Personal Glucose Monitors. Personal glucose monitors are widely commercially available and do not require laboratory skills to be operated. These devices are an interesting starting point for the development of assays targeting other analyte classes. Several studies have recently demonstrated that personal glucose sensors can indeed be adapted to detect a variety of biomolecular targets,[62] including DNA.[63] In order to specifically detect DNA, a sandwich assay was used where target DNA was first bound to a magnetic bead via a covalently attached capture strand. A second DNA strand, conjugated to invertase, binds the target. Invertase enzymatically catalyzes the

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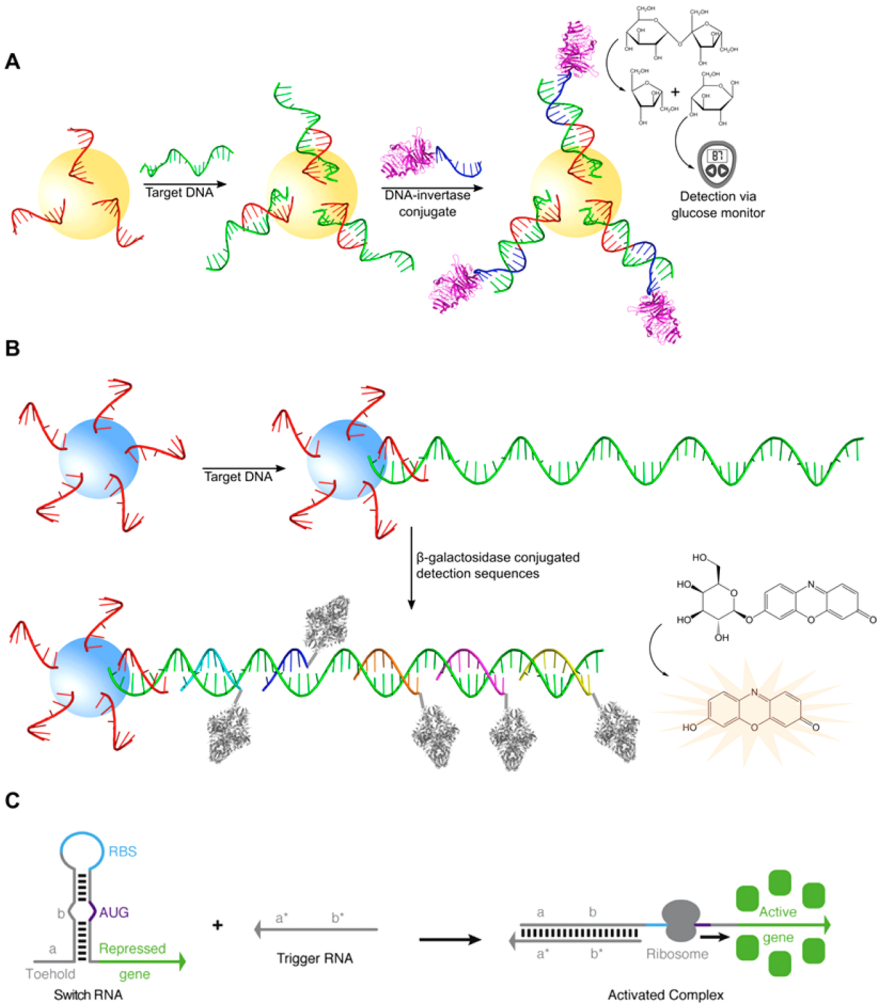

Figure 3. New methods for signal amplification. (A) Detection of DNA using a personal glucose monitor. Target DNA is bound to a bead and a second, invertase-conjugated DNA strand is added to bind the other side of the side of the target DNA. Invertase is then used to convert sucrose to glucose, which is detected by a commercially available personal glucose monitor. Adapted with permission from ref 64. Copyright 2012 American Chemical Society. (B) Reporter chemistry used in the single molecular array (Simoa) approach. DNA is captured on a magnetic bead, and a number of biotinylated DNA fragments are added to recognize additional segments of the target DNA. Streptavidin labeled β-galactosidase is then added and used to produce fluorescent resorufin. Adapted with permission from ref 65. Copyright 2013 American Chemical Society. (C) A synthetic gene circuit. Upon binding a target RNA sequence, toehold synthetic gene circuits can be turned on to produce a detectable protein product. Reprinted with permission from ref 74. Copyright 2014 Elsevier.

hydrolysis of sucrose to glucose and fructose. The glucose can then be detected using a personal glucose monitor, while both sucrose and fructose are not (Figure 3A).[64] This system allows for glucose detection at mM concentrations, the biologically relevant concentration personal glucose monitors are designed for, with a detection limit of approximately 40 pM for the target DNA. The enormous advantage of this type of approach is that it relies on instrumentation that is already widely available. Deploying this approach in a commercial-grade form is thus much more straightforward than for other approaches where custom instrumentation is required.

Digitized Optical Detection for Signal Amplification. Single-molecule array (Simoa) technology has been developed to quantitate small amounts of analytes using a bead-based approach. Genomic DNA is captured on magnetic beads, and a variety of biotinylated detection sequences bind to the genomic DNA strand.[65] A streptavidin labeled β-galactosidase enzyme is then attached to the magnetic particle to convert resorufin-β-galactopyranoside to the fluorescent resorufin.[66] Multiple biotinylated detection sequences are bound to each target DNA strand, amplifying the signal through the incorporation of multiple enzymes at each target DNA strand without steric limitations (Figure 3B). The fluorescence signal was measured on an array of fiber optics in femtoliter wells, enabling single beads to be isolated in individual wells.[67] This method of nucleic acid analysis has been used to detect double-stranded genomic DNA without purification or amplification steps at concentrations that are clinically relevant, with detection limits as low as approximately 50 aM. The digital detection approach lends itself to robust quantitation without the need for calibration, which is a tremendous advantage over approaches where more indirect signals are linked to concentrations of analyte.

Synthetic Biology-Based Approaches. In a synthetic-biology focused approach, the introduction of synthetic gene circuits has enabled designable outputs based on a specified biological input trigger.[68,69] These synthetic gene networks have been designed for a wide range of functions,[70,71] and are engineered to express protein outputs in response to biological inputs such as proteins or nucleic acids that will trigger the

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Table 3. Selected Fluidic Devices for the Automation of Nucleic Acids Analysis

microfluidic approach	representative limit of detection	detection method	speed of analysis (including sample preparation)	sample input	key advantages
Magnetic Integrated Microfluidic Electrochemical Detector (MIMED)	10 $TCID_{50}$ (viral particles)[76]	electrochemical	<4 h	virus-containing sample; all sample prep steps automated	sample-to-answer performance demonstrated with viral swabs
LAMP self-digitization chip	20 fM[83]	fluorescence	<4 h	purified DNA	isothermal amplification simplifies testing device
SlipChip	0.2 aM[86]	fluorescence	<4 h	purified viral RNA	dynamic range of 10^5–10^7
*o*Slip-DNA paper-based device	85 pM[90]	electrochemical	<1 h	purified DNA	direct, enzyme-free detection; low cost ($0.36)

circuit to be "turned on". Although initial work engineered circuits in *Escherichia coli* cells, they were recently engineered to function in cell-free environments, which eliminates potential side products in addition to simplifying their design.[72] New elements in the sensor design which create toehold switch-based circuits have enabled applications in point-of-care diagnostics by allowing for the broader design of arbitrary input RNA sequences (Figure 3C).[73] Further, synthetic gene networks have been shown to be compatible with paper-based devices, and have the capability to produce a range of protein outputs for detection, including proteins that can be used for visible output without any additional instrumentation.[74] Recent work detailed the design of a Zika sensor, demonstrating the speed at which these gene circuits can be designed to target diseases as need arises.[75] In this application, a paper-based microfluidics system was designed with the necessary cell-free lysate freeze-dried on the device, eliminating constraints for storage or shipping. When the sensor was triggered by the appropriate Zika RNA, LacZ enzyme was produced which then converted a yellow substrate to a purple product, enabling visual detection of the presence of Zika.

Gene circuits are a very attractive option for the development of biomolecular detection systems because they are highly versatile and easily reconfigured to different analytes. The major challenge with their implementation is the use of biological components; this challenge can likely be overcome with the same types of stabilization approaches that have made PCR practical.

4. FLUIDIC DEVICES FOR AUTOMATED ANALYSIS

New devices that will enable the adaptation of nucleic acid detection techniques for point-of-care applications that require complete automation is another important area that is advancing rapidly. Microfluidic devices and other approaches that allow reagents to be manipulated without the need for lab skills provide important proof-of-concept showing that new assays will be compatible with testing environments lacking lab infrastructure. Several systems that automate processing steps, reduce analysis costs, improve portability, and minimize the amount of sample and reagents needed are described below and summarized in Table 3.

Microfluidic Systems for Integrated DNA Amplification and Detection. A variety of new devices bring together target amplification technologies and detection strategies so that nucleic acid detection methods can be used in a sample-to-answer format. For enzymatic amplification approaches, sequestering reagents in a fluidic device may present a means to guard against false positives that can be caused by the presence of contaminating nucleic acids in a testing environment. Most importantly, devices that automate the fluidic manipulations required for enzymatic amplification demonstrate that nucleic acid testing can be conducted using this type of assay without the need for technical skills such as pipetting. Miniaturized devices also minimize reagent costs by shrinking the scale of amplification reactions.

Both electrochemical and fluorescence-based detection systems have been integrated with microfluidic devices automating enzymatic amplification reactions. The Magnetic Integrated Microfluidic Electrochemical Detector (MIMED)[76] allows for on-chip amplification and detection of H1N1 influenza (Figure 4A). The MIMED contains two functional units: one for capture and amplification of the sample, and one for detection. A redox-labeled DNA strand complementary to the sequence of interest is used for detection. The viral RNA and ribonucleoprotein (RNP) complex is first captured using magnetic beads conjugated to antibodies that bind the RNP. The magnetic beads are then injected into the MIMED chip, followed by PCR reagents. After amplification of the DNA sequence, the presence of the sequence of interest is detected by a decrease in the electrical current as the redox reporter is no longer able to interact with the electrode. The device is inexpensive, has a limit of detection below currently used assays, and there is very little user manipulation once the sample is loaded on the chip. The assay requires approximately 3.5 h to run, and precise temperature control is necessary.

Electrochemical detection has also been coupled with loop-mediated isothermal amplification (LAMP) of nucleic acid targets, as incubation at only a single temperature is necessary for the reaction to proceed, significantly simplifying the reaction conditions required to run the assay.[77,78] Recent work integrated LAMP and electrochemistry on a single chip to detect *Salmonella typhimurium* with a detection limit of 16 copies of genomic DNA.[79] This system exhibited important improvements over PCR in terms of time to detection and simplified temperature control. The chip was heated to 65 °C and maintained at a constant temperature to allow DNA amplification to occur, and a signal was measured in less than an hour.

Other systems have integrated LAMP on microfluidic chips where sample detection can be done visually,[80] or developed LAMP-based microfluidic devices to detect epidermal growth factor receptor (EGFR) mutations in lung cancer patients in less than an hour with visual detection.[81]

Digitization-Based Approaches for Quantitative Analysis. Microfabricated chips can also facilitate self-digitization of samples, where a single sample can be divided into many small volume aliquots for analysis without requiring manual sample handling (Figure 4B).[82] This type of approach has been developed using both LAMP[83] and PCR as amplification methods and fluorescence-based detection of the DNA sequence of interest. Digital PCR was demonstrated with an array consisting of 10^6 chambers with a theoretical dynamic

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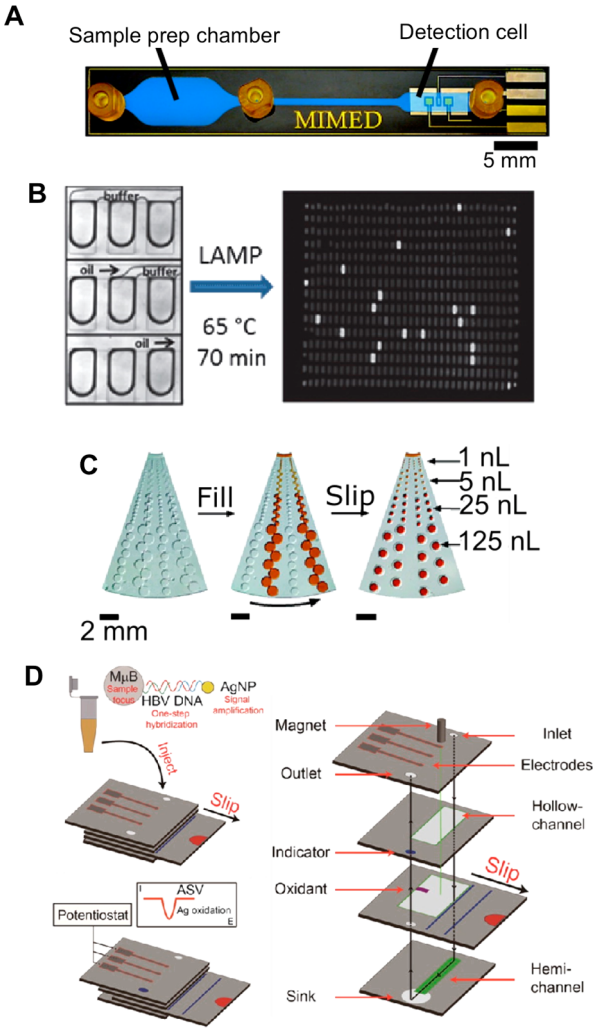

Figure 4. Microfluidics systems for nucleic acid analysis. (A) The MIMED chip allows for PCR-based amplification to first be performed, followed by electrochemical nucleic acid detection. Reprinted with permission from ref 76. Copyright 2011 American Chemical Society. (B) Self-digitization of samples allows for a large number of amplification reactions to be performed in parallel, enabling quantification of the initial target DNA. Reprinted with permission from ref 83. Copyright 2012 Royal Society of Chemistry. (C) The SlipChip can be used to digitize a sample and has been used with LAMP based detection methods. Reprinted with permission from ref 86. Copyright 2011 American Chemical Society. (D) *o*SLIP-DNA is a paper-based design with a slip layer and has been used to detect hepatitis B virus. Reprinted with permission from ref 90. Copyright 2015 American Chemical Society.

range of 10^7 using fluorescence detection of positive chambers.[84]

The SlipChip is another example of a device that digitizes samples to facilitate quantitation of target nucleic acids. This microfluidic device was designed to be preloaded with the reagents required for a diagnostic test.[85] The sample can then be loaded, and the top plate is simply moved, or "slipped", in relation to the bottom plate to mix the sample with reagents (Figure 4C). The SlipChip has been used to automate RT-PCR, and a large dynamic range was demonstrated in a study focused on analyzing the viral load of HIV and HCV.[86]

Paper Microfluidics. Since the first studies were published exploring the use of paper as a platform for microfluidic devices,[87] the use of patterned paper to create analytical devices has attracted great interest. Paper-based microfluidics are cheap to manufacture and store, and in many cases, fabrication is facile and can even be done using an inkjet printer.[88] One example of paper-based microfluidics uses the principles of origami to pattern 3D microfluidic devices on a single sheet of paper which is then folded to create the device, simplifying fabrication.[89]

An origami-based paper microfluidic device referred to as the *o*Slip-DNA was recently described that features a sensitive detection approach that is enzyme-free. A three-strand DNA sandwich assay was used to link silver nanoparticles to magnetic beads, and the presence of silver was detected after oxidation to soluble Ag^+, followed by electrodeposition on the electrode.[90] The *o*Slip-DNA was shown to detect hepatitis B virus (HBV) DNA with a limit-of-detection of 85 pM (Figure 4D).

Fluidic devices are presenting powerful solutions to the automation of nucleic acid detection assays. By demonstrating that assays can be performed without human intervention or in formats where manual manipulations are not required, these devices show that nucleic acid analysis can be performed anywhere that molecular-level information is required. The development of versatile solutions for the processing and analysis of varied clinical specimens (blood, swabs, urine, etc.) represents an important future direction. Many of the systems reported thus far are tailored to one application or sample type; in a clinical setting devices must be multipurpose to be useful enough to justify their routine use.

5. CONCLUSIONS

Recent advances in the sequence-specific detection of nucleic acids now provide a number of options for rapid, cost-effective diagnostic methods. Limits of detection for direct-detection approaches are beginning to reach ranges that are clinically relevant without additional purification or amplification steps. Nanomaterials and new signal amplification approaches are playing important roles in the development of advanced DNA and RNA sensors, and work with fluidic devices is demonstrating that assays can be automated to be made practical for clinical use.

That said, with all of the recent progress, there are few DNA detection devices that are commercialized and available for clinical use. This relates to the difficulty of translating research-grade technologies into products, and the complexity of obtaining sufficient sensitivity and specificity in clinical samples. However, with the abundance of new systems that are candidates for high-performance nucleic acid analysis, these challenges should be overcome. As fluidics devices, and especially cost-effective paper-based devices gain prevalence, it is expected that DNA- and RNA-based molecular diagnostics will become routine.

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Notes

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