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Ultra-fast electronic detection of antimicrobial resistance genes using isothermal amplification and Thin Film Transistor sensors

Chunxiao Hu[a], Sumit Kalsi[a], Ioannis Zeimpekis[a], Kai Sun[a], Peter Ashburn[a], Carrie Turner[b], J. Mark Sutton[b], Hywel Morgan[a,*]

[a] Department of Electronics and Computer Science, and Institute for Life Sciences, University of Southampton, UK
[b] National Infections Service, Public Health England, Porton Down, UK

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ABSTRACT

A low cost thin-film transistor (TFT) nanoribbon (NR) sensor has been developed for rapid real-time detection of DNA amplification using an isothermal Recombinase Polymerase Amplification (RPA) method. The semiconductor chip measures DNA amplification through a pH change, rather than via fluorescence. The utility of the method was demonstrated by amplifying CTX-M and NDM, two genes that confer bacterial resistance to cephalosporins and carbapenems, respectively. It is shown that this approach provides extremely fast and sensitive detection. It can detect < 10 copies of the gene in genomic DNA extracted from *E. coli* or *K. pneumoniae* clinical isolates within a few minutes. A differential readout system was developed to minimize the effect of primer-dimer amplification on the assay. The simple device has the potential for low cost, portable and real-time nucleic acid analysis as a Point of Care device.

1. Introduction

There is a drive to develop fast and simple diagnostic tools to aid in the identification and diagnosis of a range of diseases. Amongst infectious disease, antimicrobial resistance (AMR) is rapidly becoming a global threat as pathogens evolve new resistance strategies, potentially making even simple infections difficult to treat (O'Neill, 2016). The production of extended spectrum β-lactamases (ESBLs) in Gram-negative bacteria is an example of a problem that has spread rapidly in the last decade fuelled by the overuse of antibiotics. CTX-M is the most prevalent ESBL globally, conferring resistance to key cephalosporinantibiotics such as cefotaxime. The $bla_{\text{CTX-M}}$ gene, which encodes a number of different amino acid variants, CTX-M-15 being the most common in the UK, has spread rapidly across at least 26 bacterial species residing in both nosocomial and community environments (Zhao and Hu, 2013). NDM-1 is a gene which mediates resistance to carbapenems, one of the last drug types which is effective against multidrug resistant Gram-negative bacteria. This resistance gene was originally identified in 2008 and has spread widely around the world, facilitated by its presence on a highly promiscuous plasmid which can be moved from one bacterial species to another. Organisms carrying $bla_{\text{NDM-1}}$ plasmids are also often multidrug resistant, with the plasmid often carrying resistance genes for fluoroquinolones, aminoglycosides and other antibiotics. Given the rapid dissemination of resistance genes and the emergence of strains which are commonly multidrug resistant, there is a clear need to develop rapid and sensitive tests to determine antibiotic resistance, both as part of the diagnosis and management of infection and to provide a rational basis for the appropriate prescription of antibiotics.

Current clinical tests are based on bacterial cell culture that is very slow, or on PCR based approaches that are based in centralised labs requiring expert users and specialized equipment. In both cases, analysis cannot easily be done at Point of Care, thereby delaying treatment and potentially leading to inappropriate antibiotic therapy. The most rapid, sensitive and specific molecular assays are based on nucleic acid amplification. These are used to detect specific genes of interest that confer resistance, for example those that encode for ESBLs and carbapenemases. PCR is the method of choice for central lab based methods and has also been used in Point of Care, Lab on Chip systems (Cho et al., 2007; Hua et al., 2010; Lee et al., 2006; Norian et al., 2014). Isothermal DNA amplification techniques, see (de Paz et al., 2014; Li and Macdonald, 2015; Yan et al., 2014) are particularly attractive for use at the Point of Care since it does not require thermal cycling equipment, thus greatly simplifying the technology. These reactions also operate at lower temperatures than PCR (e.g. 40–60 °C), thereby requiring less power and eliminating the need for complex control systems. Some isothermal techniques require an initial DNA denaturation step at an elevated temperature but many others including loop mediated isothermal amplification (LAMP), helicase-dependent amplification (HDA), and Recombinase Polymerase Amplification (RPA) can

* Corresponding author.

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amplify DNA at a single temperature thus simplifying the system. Isothermal techniques are also generally faster, for example RPA can produce a result in 15 min; although a fast PCR (1 min) technique has been developed it uses complex high-speed heating and cooling technologies (Farrar and Wittwer, 2015). Generally the readout for these assays is optical and uses either real time fluorescent probes, or colorimetric methods for simpler point of care diagnostic devices.

RPA is gaining popularity as a simple and robust method of amplifying DNA (Daher et al., 2016). It is particularly amenable for the rapid and cost-effective identification of pathogens as it requires little sample preparation and shows little inhibition from a wide range of body fluids including serum, stools and urine (Daher et al., 2016). Unlike LAMP that needs between 4 and 6 primers, RPA only requires 2 primers. RPA uses recombinase enzymes for primer annealing and unwinding DNA. Real-time amplification of product is usually monitored using a fluorescent readout that uses an exo probe. This is a long complimentary oligonucleotide that has a tetrahydrofuran (THF) moiety located between a fluorophore and a quencher. The exonuclease III cleaves the THF once the probe has bound to the target sequence emitting fluorescence. RPA is relatively fast (typical times to positivity in the range 10–15 min), and operates at a relatively low temperature (37–41 °C).

To simplify analysis, there has been interest in monitoring DNA amplification electronically through measuring pH change. In a PCR reaction, a single nucleotide incorporated into a strand of DNA results in the release of a single proton which can be used to measure DNA amplification via pH changes (Toumazou et al., 2013; Zhang et al., 2014a). Recent work has also shown how conductivity changes can be used to measure amplification of DNA by LAMP (Zhang et al., 2015) using pH-sensitive dyes (Tanner et al., 2015).

The ion-sensitive field effect transistor (ISFET) is a simple solid state device that accurately measures pH [(Bergveld, 1970; Caras and Janata, 1980; Kimura et al., 1989; Lee et al., 2009; Schoning and Poghossian, 2002) and has been used to detect pH changes following nucleotide incorporation in primer extension assays (Bergveld, 2003; Purushothaman et al., 2006; Sakurai and Husimi, 1992; Sun et al., 2014; Toumazou et al., 2014). Toumazou et al. (2013, 2014) has pioneered the use of CMOS ISFET technology for DNA analysis. A single CMOS chip can perform DNA amplification and detection by monitoring pH change for PCR and isothermal LAMP. The chip contained embedded heaters, temperature sensors and control circuitry, with an array of ISFET pH sensors for readout. The IC platform reduces the size and cost of measurement, eliminates the use of functionalization steps and biomarkers like fluorescent dye, and provides direct electrical readout of DNA amplification. However the CMOS chip is still relatively expensive, and in their isothermal LAMP measurements, the time to positivity was in the range of 15–20 min for 10 ng genomic DNA (approximately 3000 copies).

With the aim of both accelerating the time to result and simplifying the readout method we have combined an isothermal nucleic acid amplification assay with pH based electrical readout using simple low-cost Thin Film Transistor (TFT) sensors. RPA was used to identify target DNA extracted from antimicrobial resistant microorganisms. TFT sensors are extremely simple and inexpensive to manufacture making them ideal candidates for electronic disposable sensors. Amplification was performed and monitored on the TFT chip, with direct electrical real-time readout. The system was used to demonstrate rapid and selective amplification of two different target sequences to identify AMR resistance genes from *E. coli* and *K. pneumoniae*. Time to positivity was under 3 min for 100 copies as compared to 15–20 min for conventional benchtop methods with fluorescent readout.

2. Materials and methods

2.1. TFT Device fabrication and architecture

TFT is an attractive alternative to CMOS-based semiconductor technologies and is widely used for the manufacture of displays as used in consumer electronics products. These are mass manufactured as large sheets of glass (2.88 m×3.13 m for 10th generation) giving a very low cost per unit substrate area. Therefore, a TFT sensor offers the prospect of extremely low cost disposable systems that could include all functionalities from sample preparation to DNA amplification and assay readout. The TFT NR sensor is essentially a field effect transistor with the metal gate replaced by solution. The surface of any metal oxide always contains hydroxyl groups (Bergveld, 2003) – silanol groups in this study. These groups may donate or accept a proton H^+ from the solution, leaving a negatively charged or a positively charged surface group respectively depending on pH. This change of the surface potential modifies the current in the semiconducting channel, which is the measured signal.

The TFT silicon NR sensors were fabricated using a very simple three-mask fabrication method that has been described previously (Sun et al., 2014). Briefly, a 45 nm amorphous silicon film was deposited by Plasma Enhanced Chemical Vapour Deposition (PECVD) and in-situ doped using phosphorous. A 8.5 nm thin gate oxide was then grown at 900°C in dry O_2. This process also crystallised the amorphous silicon into polycrystalline silicon and fully activated the phosphorus dopant. A first mask was used to define the NR patterns from polysilicon. Ohmic contacts from TiN were made using a second mask. TiN is a hard, resilient material that allows the biosensor chip to be directly interfaced with a standard micro pitch board-to-board connector to a printed circuit board without requiring wire bonding. A third mask defined the encapsulation layer and the sensing window in contact with the liquid.

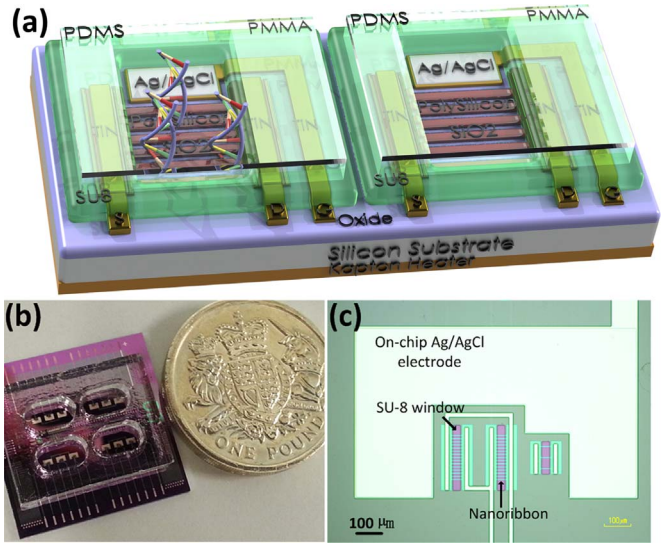

Fig. 1. Thin Film Transistor Nanoribbon (a) Schematic diagram of the TFT sensor showing the measurement configuration (not to scale). Two NR transistors are used in a differential configuration. Each sensor has an integrated Ag/AgCl reference electrode. Both contain RPA chemistry and primers, but only one has target DNA; (b) Photograph of the device. Each Si chip comprises 12 separate sensors in four PMMA wells that contain the sample. To prevent evaporation, the wells were covered with a thin PDMS lid; (c) Microscope image of a single NR sensor which is made of 30 nanoribbons connected in parallel, each 40 µm long. Also shown are the metal tracks that connect to the two sets of nanoribbons. The entire Si chip is covered in SU8, except for small windows that exposes the sensing area as shown in the figure. The entire region is also surrounded on three sides by a large integrated Ag/AgCl reference electrode.

The final fabricated device is shown in Fig. 1. The chip has an array of identical sensors, so two identical sensors can be used for differential measurements of DNA amplification. The NR transistor has two gates. The top gate is a liquid gate controlled by applying a potential to the integrated on-chip Ag/AgCl reference electrode (Fig. 1a). The bottom gate is the back of the Si substrate, which was grounded for all measurements described in this paper. The top gate dielectric is in contact with the liquid and made from silicon dioxide. Fig. 1c shows a microscope image of a single NR sensor which is made of 30 nanoribbons connected in parallel, each 40 μm long. An SU8 layer defines a small sensing window (Fig. 1c). The SU8 window is confined within a well, fabricated using holes cut in a thin PMMA (Poly(methyl methacrylate)) sheet (Fig. 1b), defining a volume of 50 μl. The temperature of the chip was controlled with a thin flexible Kapton heater (OMEGA Engineering Inc; UK) stuck to the bottom of the chip – Fig. 1a. In principle simple resistive heater could form part of the chip fabrication process but this was not done in this work. A K-type thermocouple (RS Components Ltd; UK) and a PID (proportional–integral–derivative) controller (RS Components Ltd; UK) was used to measure and control the temperature. A dual-channel picoammeter/voltage source (Model 6482, Keithley) applies the required voltages and measures the transistor source-drain current, via a custom made interface jig (Sharp Laboratories of Europe).

2.2. Isothermal Amplification

2.2.1. DNA template preparation

Strains expressing the target resistance genes (*Escherichia coli* NCTC 13441 and *Klebsiella pneumoniae* NCTC 13443) were isolated from human clinical sample and obtained from the National Collection of Type Cultures at Public Health England. The genome of NCTC 13441 has been sequenced (EBI accession number SAMEA2709038) and is known to contain genes for ESBL CTX-M-15, while NCTC 13443 (EBI accession number SAMEA2742597) has genes for ESBL CTX-M-15 and the carbapenemase NDM-1. Liquid cultures were obtained by transferring a single bacterial colony from Tryptone Soy Agar (TSA) plates into Tryptone Soy Broth (TSB). The culture was incubated overnight at 37 °C. DNA from the overnight culture was extracted using the DNeasy Blood and Tissue Kit (Qiagen, UK) following the protocol described by the manufacturer. The eluted DNA was quantified using Qubit fluorometer using DNA assay reagents. Genome copies were calculated from the DNA concentration using an in-house copy number calculator, although many are freely available online.

2.2.2. RPA primer and probe design

Primer sets spanning the $bla_{\text{CTX-M-15}}$ gene and $bla_{\text{NDM-1}}$ gene were designed and tested in multiple combinations. RPA exo probes (for benchtop experiments) containing a CY5 fluorophore (for $bla_{\text{CTX-M-15}}$ gene) or Quasar ® 670 fluorophore (for $bla_{\text{NDM-1}}$ gene) were designed and evaluated using TwistAmp exo kits (TwistDx, UK). The sequences for the primers and probes used in the study are available on request from Public Health England.

2.2.3. Benchtop RPA assay

RPA operates in the temperature range of 37–41 °C. It uses three proteins: recombinase, single-stranded DNA-binding protein (SSB) and polymerase (Piepenburg et al., 2006). This forms complexes with two primers specific to the target gene that scans the DNA for complementary sequences. A T4 polymerase, Bsu, extends the primers, resulting in two copies of the original DNA. The amplification reaction is shown schematically in Fig. 2a. Amplification is usually monitored in real time using fluorescent exo-probes that are complementary to the amplified DNA fragment, see Fig. 2b. The amplitude of the fluorescence signal is proportional to the amount of amplicon produced in the RPA reaction. For analysis by gel electrophoresis, the TwistAmp exo kits cannot be used as they contain exonuclease III which digests the DNA.

Benchtop RPA reactions were performed using the TwistAmp exo kits, following manufacturer's protocols. Briefly, lyophilized RPA proteins were reconstituted with a mixture of rehydration buffer, forward and reverse primer, probe and sample. The rehydration buffer was as supplied by the manufacturer and was not changed in any way. Every 50 μl reaction contained 0.42 μM of each primer, 0.12 μM probe and 14 mM of magnesium acetate. After initiating the reaction by addition of magnesium acetate, the reaction was mixed rigorously and transferred to a GloMax microplate reader (Promega, UK) set at a temperature of 39 °C. Fluorescence was measured every minute for 30 min. The time to positivity for each sample was measured from the time at which the fluorescence exceeds the threshold calculated by three times the standard deviation of negative controls.

2.3. Electrical measurement of RPA amplification

For assays such as PCR and LAMP, the pH change measured during DNA amplification occurs because of the incorporation of a single nucleotide into a DNA strand, thus releasing protons and acidifying the suspending electrolyte, which must be weakly buffered (Toumazou et al., 2013). RPA has an additional element in that it includes a recombinase that binds cooperatively to the primer (oligonucleotides) in the presence of adenosine triphosphate (ATP). The resulting nucleoprotein complex actively hydrolyses ATP to produce adenosine diphosphate (ADP) and an inorganic phosphate, orthophosphate (Pi). The process of ATP conversion to ADP also results in the generation of H^+ (see Fig. 2c). The RPA buffer contains phosphocreatine and creatine kinase which results in the recycling of ADP to regenerate the ATP needed by the recombinase enzymes. This process may use up the free H^+ ions released by the ATP hydrolysis but not those generated by base addition to the growing DNA chain.

Electrical measurements were performed in differential mode using two TFT NR sensors, with a measurement chamber containing RPA, primers, and DNA, and a reference chamber which had only primers and deionized water (No template control (NTC)). Prior to DNA amplification, the TFT NR sensors were incubated with 3% BSA (Sigma) for 30 min to minimize any potential non-specific binding of proteins and DNA. The chips were heated to 39 °C and immediately prior to measurement, magnesium acetate was added to the RPA solution (target DNA and primers), vigorously mixed with a pipette and aliquoted onto the sensor surface. The reaction was monitored by recording the source-drain current (with source-drain voltage V_{SD}=0.1 V; liquid gate voltage V_{lg}=0.05 V) as a function of time. The pH of the solution was then determined from the normalized Source-Drain current. Measurements of DNA amplification were performed in differential mode eliminating common mode effects such as temperature fluctuations and electrical signal drift. One TFT NR was used as a reference sensor, which was exposed to all the RPA components without the DNA template. The second sensor was identical except that it included the target DNA.

3. Results and discussions

3.1. Electrical characteristics

The pH sensitivity of a NR chip was first determined from liquid gate sweeps by monitoring the current through the source-drain ($I_{SD}V_{lg}$) using calibrated pH buffers (Sun et al., 2016). Four different pH buffers (pH=9.0, 7.0, 5.0 and 3.0) were used to characterize the electrical response of the devices at the same temperature as the RPA reaction, 39 °C. The NR is n-type and therefore the source-drain current I_{SD} increases with decreasing pH. The transconductance $gm=\partial I_{SD}/\partial V_{lg}$ was used to quantify the device response to pH change. This was determined from the linear region of the $I_{SD}V_{lg}$ curve and was found to be nearly constant at ~2×10^{-7} A/V for the four pH values (ESI Fig. S1). The threshold voltage shift $\Delta V_{th}=\Delta I_{SD}/g_m$ was extracted from

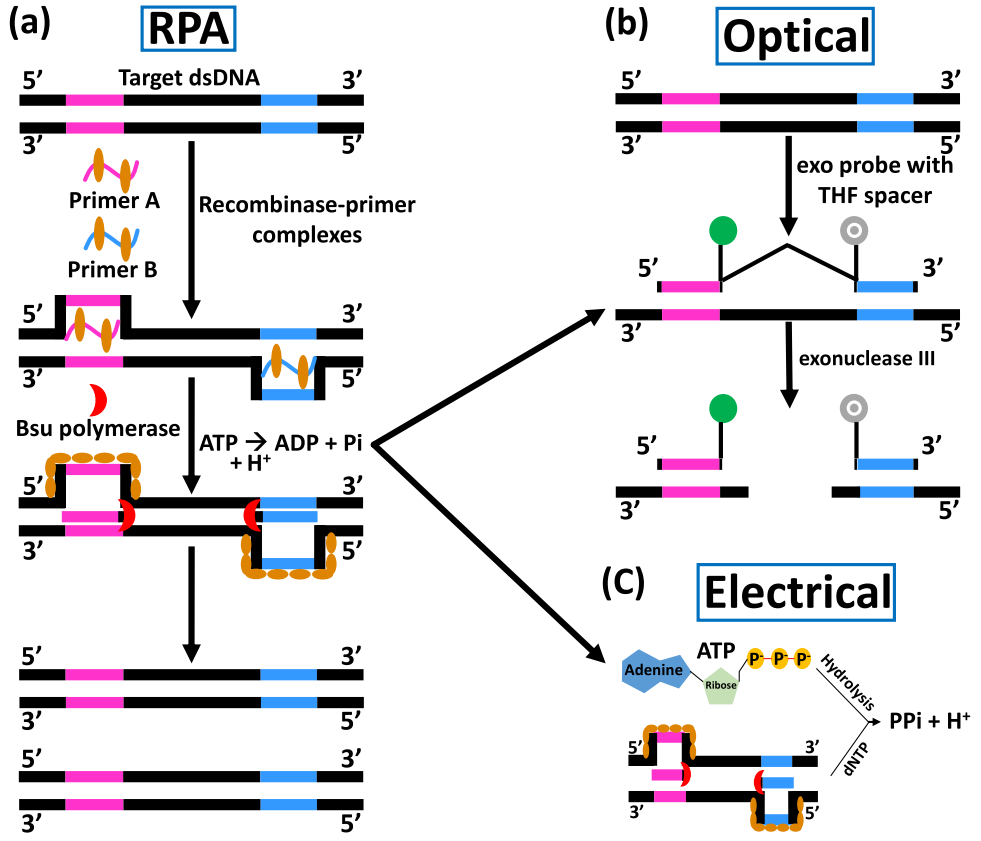

Fig. 2. The Recombinase Polymerase Reaction (a) Illustration showing the steps of the RPA reaction (adapted from Piepenburg et al. (2006)). Double-stranded (ds) template DNA hybridises with recombinase–primer complexes. Primers are extended by the Bsu polymerase in D-loop structure from both 5′ and 3′ directions. This results in two copies of the original ds DNA. During amplification, ATP is hydrolysed to ADP releasing a proton. (b) Optical detection: the exo- fluorescent probe anneals to a complementary sequence on the template DNA. When bound, an exonuclease III digests the tetrahydrofuran (THF) spacer on the probe providing a real-time fluorescence signal which is proportional to DNA concentration. (c) Electrical detection: possible sources of protons during DNA amplification from (a) nucleotide incorporation and (b) chemistry related to hydrolysis of ATP leading to generation of PPi.

the $I_{SD}V_{lg}$ curves for the pH range used. Electrical characterization shows that the sensitivity of the sensors is sub-Nernstian as widely reported for SiO_2 gate dielectrics (Sun et al., 2016) at 32 mV/pH.

3.2. Real-time DNA amplification and detection using RPA

Purified DNA from two organisms was used to demonstrate the applicability of the developed platform for different AMR genes detection: *E. coli* (*NCTC 13441, containing* $bla_{CTX\text{-}M\text{-}15}$ gene) and *K. pneumoniae (NCTC 13443, containing* $bla_{CTX\text{-}M\text{-}15}$ and $bla_{NDM\text{-}1}$). For NCTC 13441, presence of $bla_{CTX\text{-}M\text{-}15}$ in the sample was tested while for NCTC 13443, $bla_{NDM\text{-}1}$ was detected. The sensitivity of the device was tested for 0, 9, 90, 900 and 9000 copy numbers in a 30 µl chamber (0–56 pg). Fig. 3a shows the NR response (normalized source drain current divided by *gm*) for the two different sample chambers (reference and sample) monitored simultaneously for the CTX-M-15 sample. The reference chamber is the No Template Control (NTC) and contains RPA chemistry and primers but no sample DNA (NTC & primers). The sample chamber contains DNA at an initial concentration of 9000 copies. The reference chamber was first filled, followed by the sample chamber (with a delay of a few seconds). The difference signal from these two chambers is the final result plotted in Fig. 3b. Measurements were also performed using the genomic DNA containing $bla_{NDM\text{-}1}$ see Fig. 3c. Primers were always present in the reference chamber. The same DNA was also amplified using the RPA exo-kit using a benchtop fluorescence plate reader. Similar amplification curves were obtained – see Fig. S3.

Analysing the data in Fig. 3a with the previously determined sensitivity of the device, gives the equivalent pH change for the RPA reaction for 9000 copies to be approximately 0.91 pH units. This pH change was corroborated using a commercial pH microelectrode (Cole-Parmer, UK) that was used to monitor the pH of an RPA reaction in the NR well at the same time as the TFT sensor. The pH change for a reaction occurring in a microplate well was also measured. For 9000 copies, the pH of the solution in the NR well as measured with the pH microelectrode changed by a similar amount, with ΔpH=0.93, whilst the change was slightly larger in the microwell; ΔpH=1.02. Fig. S2. This may be due to a slightly different buffering capacity in the NR system compared with the plastic microwell, which has a higher volume and surface area (plastic rather than SiO_2). The pH change in the reference well was also measured with a commercial reference electrode and found to be less than 0.1 pH change for the same reaction.

As the initial concentration of the primers and the buffer are known, the maximum pH change for the RPA reaction can be calculated from the Henderson-Hasselbalch equation. Unlike previous work (Toumazou et al., 2013), this assay was performed using commercial RPA kits, which contains 50 mM Tris buffer (pH 8.3) with 50 mM phosphocreatine and 2.5 mM ATP. Assuming all the primers are used up to make the amplicon then the maximum change in proton concentration due to H+ release during DNA synthesis (of 9000 copies) would be 0.42 µM x2 x 260(bp)=0.22 mM, which will hardly affect the pH of the solution given the buffering capacity of the reagents. The question remains as to why the pH of the solution changes by such a large amount. In addition to the production of protons, each base addition to the growing amplicon will release one molecule of inorganic pyrophosphate (PPi). Coincidentally this can be detected directly by precipitation of the Mg^{2+} salt of PPi in the LAMP isothermal assay. The PPi is also produced at very high level within the RPA assay, which may

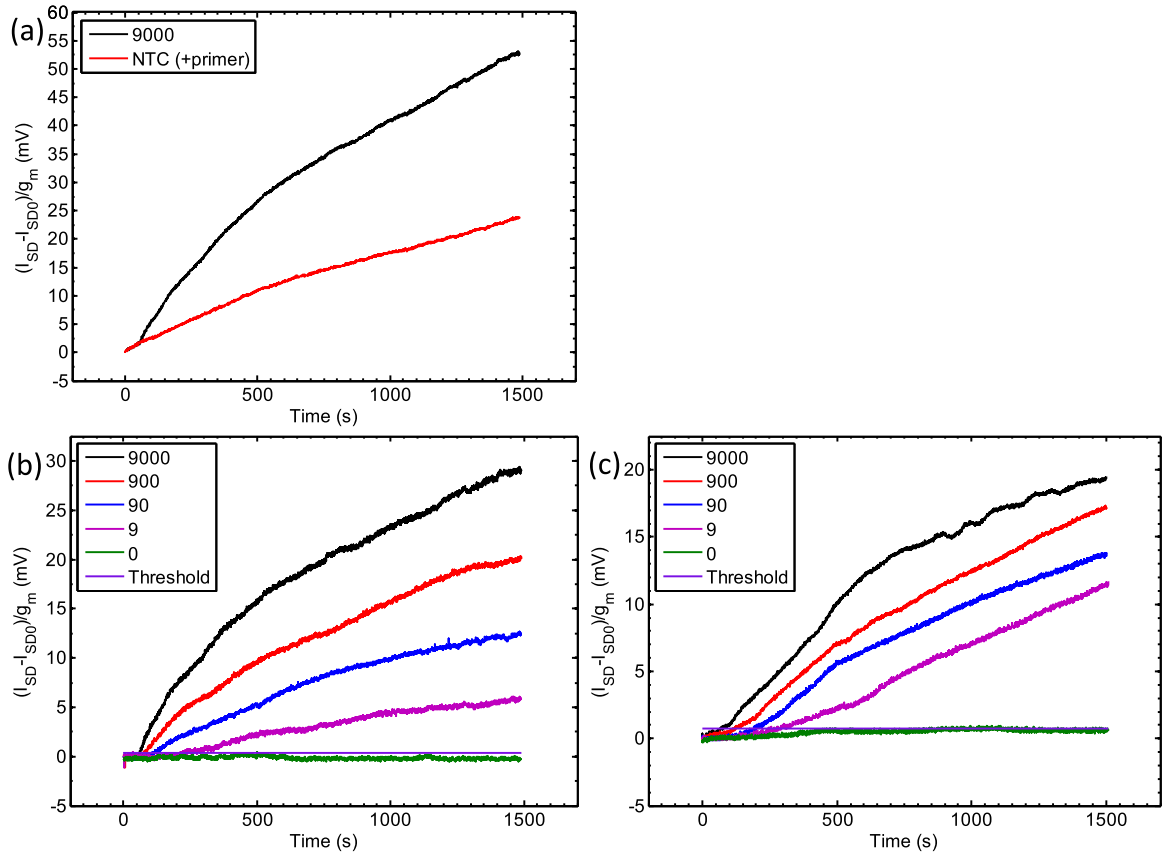

Fig. 3. Isothermal RPA DNA amplification curves measured with the TFT NR sensor. (a) Single-ended measurement of normalized current (I_{SD}-I_{SD0}/g_m in mV) for 9000 initial DNA copies of NCTC13441 *E. coli* containing $bla_{CTX\text{-}M\text{-}15}$ gene in the detection chamber (black line); NTC (+primers) in the reference chamber (red line); (b) Differential amplification data (black curve minus red curve in (a)) for 5 different initial DNA copy numbers containing $bla_{CTX\text{-}M\text{-}15}$. The threshold line is drawn as 3 X S.D. NTC. (c) Similar titration data for genomic DNA extracted from NCTC13443 *K. Pneumoniae*, tested for the presence of $bla_{NDM\text{-}1}$ gene. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

lead to changes in the pH. The dissociation chemistry of PPi in solution is also complex with a variety of species ($H_3P_2O_7^{1-}$, $H_2P_2O_7^{2-}$, $HP_2O_7^{3-}$, and $P_2O_7^{4-}$) present in varying amounts with proportions being affected directly by pH (Credo et al., 2012; Zhang et al., 2014b, 2015). The contribution of this mixture of species and its known propensity to precipitate metal ions may also contribute to the measured values. PPi conversion into ATP has also provided the basis for real-time read-out of isothermal amplification methods (Gandelman et al., 2010) and is being explored as the basis of alternative rapid detection methods (Fischbach et al., 2017).

3.3. Primer-dimer amplification during RPA

Measurement of the pH change of the RPA reaction in the absence of target DNA shows a significant change in pH as seen in Fig. 3a. This can be attributed to the creation of primer dimers that can be readily identified by gel electrophoresis. Normally, the exo-kit RPA mitigates this problem through the use of sequence specific fluorescent hybridization probes. Primer dimer creation can be a problem in RPA reaction and the effect of this on the signal was minimized using the dual-sensor approach.

To verify that this signal change in the reference chip is indeed due to primer amplification rather than temperature change or electrical signal drift, the primers in the reference chamber were replaced with D.I water, i.e. the reference chamber had neither DNA template nor primers (NTC(-primer)). An RPA reaction was carried out in the same way with 9000 initial DNA copies containing the $bla_{CTX\text{-}M\text{-}15}$ gene in the sample chamber, and the electrical signal is shown in Fig. 4a. Note that this data is from a new device and shows a smaller shift of only 30 mV (compared with 50 mV for Fig. 3a) for the same pH change. The signal from the reference chamber does change but this is much smaller than when primers are present (Fig. 3a). Two further plots are shown in Fig. 4a, which are signals from NR sensors with only PBS buffer in the reference well. These plots show that the change in the control well is entirely due to electrical drift in the system, which is eliminated through the use of the differential system. The differential signal from the detection and reference chamber for a range of DNA concentrations without primers is shown in Fig. 4b. Although the overall change in pH is similar to the data shown in Fig. 3, the shapes of the plots are different. Also the “no-DNA” plot (green) indicates that primer-dimerisation is occurring, leading to an increase in pH which is not seen in the fully differential measurements.

The products of the RPA assay were identified from gel electrophoresis using a Bioanalyzer (Agilent 2100). The DNA was amplified in the NR sensor sample well and the products analyzed. Three individual RPA products were examined, (2) -primer NTC, (3) +primer NTC and (4) 9000 copies as illustrated schematically in Fig. 5a. The gel electrophoresis image is shown in Fig. 5b. No DNA was amplified without target DNA and specific primers; lane (3). This lane also shows several weak bands when only primers are present (no target DNA). For the complete RPA reaction with 9000 initial target DNA copies. As shown in lane (4), a clear strong band is observed at around 260 bp (the amplicon size), with two weaker bands also present, showing that not only the target DNA (strong band), but also the primers were amplified (two weaker bands) during the RPA process. The presence of these bands corroborates our assumption that the pH change due to these extra primer bands is effectively cancelled out using the differential measurement configuration.

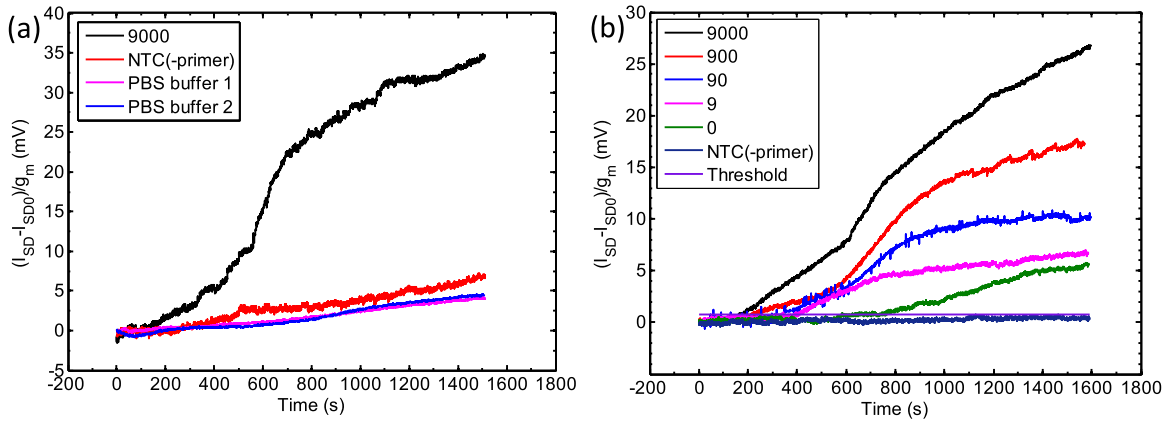

Fig. 4. Electronic measurement of DNA amplification without primers in the reference chamber. (a) Measurement with RPA enzymes, primers and 9000 initial DNA copies containing the $bla_{CTX\text{-}M\text{-}15}$ gene (black line). The NTC(-primer) data (red line) is from the reference chamber that has no DNA (NTC) and no primers (only RPA enzymes). Two further data sets (blue and pink) for a well containing only PBS are shown demonstrating that the change in signal observed from the NTC (no sample DNA) is due to electrical drift in the system. (b) Differential data (black minus red) for 6 different initial DNA copy numbers (without primers in reference chamber). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

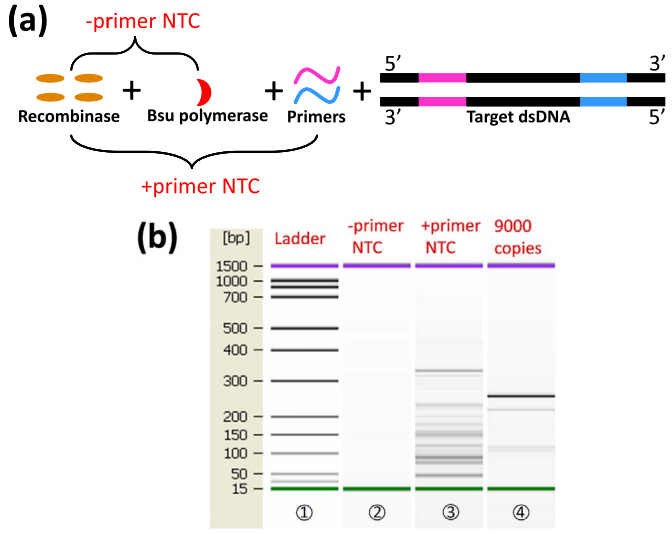

Fig. 5. Gel electrophoresis results. (a) Schematic diagram showing the different components in the RPA assay; (b) image of a gel electrophoresis results (from Agilent Bioanalyzer) for the RPA products from different experiments. No band was observed for −primer NTC (②), many weak bands for +primer NTC (③), and one strong band (with two weaker bands) for 9000 copies, with primers (④).

In a conventional RPA, the amount of DNA present in the sample can be quantified from an estimate of the time at which the fluorescence signal crosses a threshold (Kalsi et al., 2015; Piepenburg et al., 2006), known as the time to positivity (TTP). The threshold of detection is usually defined as 3 times the standard deviation of the negative control. A plot of TTP against the logarithm of the DNA copy is shown in Fig. 6 for both the bench top assay and the TFT assay demonstrating the log-lin relationship for the RPA assay.

As shown by the figure, the assay performed on the TFT sensor is significantly faster that for the fluorescent assay with the exo-probe. Compared to bench top RPA measurement (Kalsi et al., 2015) the TTP measured electrically with the NR sensors is significantly faster, reduced from 17 min to 3 min for 900 copies (Fig. 6a). Interestingly amplification of NDM-1 using the exo-probe is both slower and less sensitive than for the CTX-M. However, both genes have approximately the same sensitivity and TTP when measured electronically pointing to difference in the kinetics of the exo-probe. The LoD for the NDM-1 assay using the benchtop assay was 10^4 copies, but as few as 10 copies could be detected on-chip in 5 min.

4. Conclusion

This work demonstrates a novel method for real-time on-chip isothermal DNA amplification using a low cost TFT NR sensor with high speed and high sensitivity. DNA amplification was performed

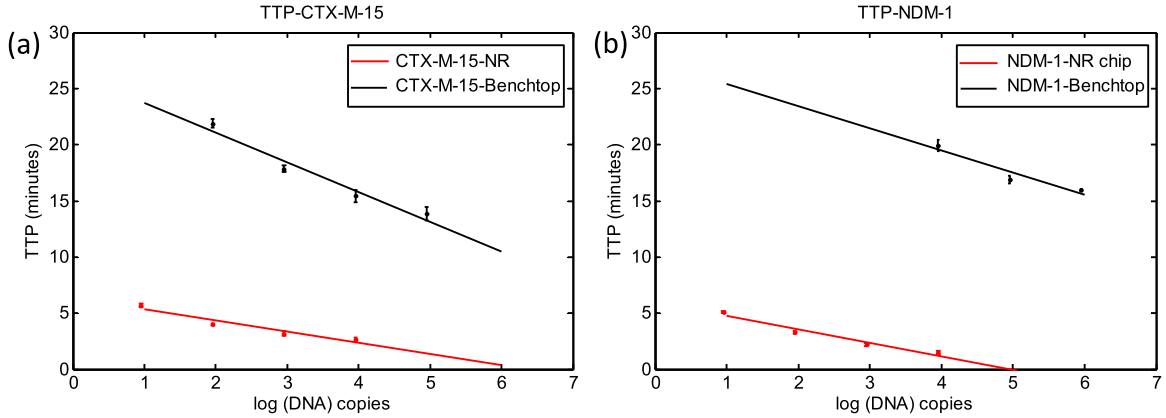

Fig. 6. Time to positivity (TTP) plots for benchtop and TFT NR chip. (a) NCTC13441 *E. Coli* DNA containing $bla_{CTX\text{-}M\text{-}15}$ gene (black line is Benchtop and red line is NR chip). (b) NCTC13443 *K. Pneumoniae* DNA tested for $bla_{NDM\text{-}1}$ gene (black line is Benchtop, red line is NR chip). Note the lower LoD for this assay on benchtop. The data consists of three replicates from two devices for each measurement, along with standard error of mean (S.E.M.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

using Recombinase Polymerase Amplification (RPA). The effect of electrical drift in the sensors and the consequences of primer-dimer amplification in the RPA were minimized using a differential measurement. RPA of two different AMR genes (encoding for CTX-M-15 and NDM-1) demonstrated that the system is faster and more sensitive than conventional fluorescent exo-probe readout without the need for complex fluorescent or other optical readout, and the DNA is quantified by measuring the TTP. The measured pH change is considerably larger than that estimated from nucleotide incorporation alone. The exact mechanism for the enhanced pH change is unclear at this stage, but possible linked to the generation of PPi via the unique enzyme chemistry of the RPA reaction. This requires further investigation that is outside the scope of this paper. The use of low-cost TFT technology for electronic readout provides new opportunities for quantitative yet simple diagnostic solutions. The transistors are easy and inexpensive to manufacture and could be simply incorporated into more complex fluid processing platform including digital microfluidic systems or even paper diagnostics.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2017.05.016.

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