INTEGRATED 'LAB-ON-A-TRANSISTOR': WITH DROPLETS-IN-AIR FOR PARALLEL NANOLITER REACTIONS

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ABSTRACT

We aim to take 'lab-on-chip' technology further by introducing the concept of a 'lab-on-transistor'. In this methodology, laboratory operations, such as heating, cell lysis, and detection, are performed on a single transistor instead of on an entire microchip. To demonstrate this concept, we developed a heating technique that allows transistors to act as electrically addressable, individual heating units. We have coupled the transistor heaters with placement of sub-nanoliter droplets to create individual heated reaction volumes. Under this configuration transistors become highly localized heater/sensors capable of high-speed thermocycling (>25°C/s) of <1nL reactions with potential for electrical detection of biological analytes.

KEYWORDS

Droplets, Field effect transistor, PCR, parallel, denaturation, electroporation.

INTRODUCTION

The crisis in the management of infectious disease for the developed world and in the developing world requires rapid, easy to use, integrated, and inexpensive diagnostic devices for the detection of bacterial and viral agents of infectious diseases. The urgency of this critical need cannot be over-emphasized, since millions will benefit from the use of rapid diagnostic technologies. Specifically, nucleic acid-based methods are still considered the gold-standard for detection and identification of microorganisms and viruses due to their high specificity and selectivity as compared to antibody-based assays. The recent technological advances in microfluidics and micro/nanotechnology present new opportunities for development of small, sensitive, single-use, point-of-care "Lab on Chip" (LOC) diagnostic devices that are capable of providing a rapid analysis of nucleic acid amplification for global health applications. Specifically, Nanowire field effect transistors (FETs) have been widely used as sensors for detection of biological products including small molecules, proteins, and nucleic acids. [1] Biological FET's provide high sensitivity, small size, portability and low cost making them an attractive option for 'lab-on-a-chip' applications. [2]

To take current LOC techniques one step further, we propose to develop the concept of a "Lab-on-a-Transistor" for point-of-care testing that will be capable of: (i) cell capture and thermal lysing, (ii) ultra rapid techniques for performing nucleic acid amplification and (iii) rapid, electrical detection of the amplified products on silicon transistors (Figure 1).

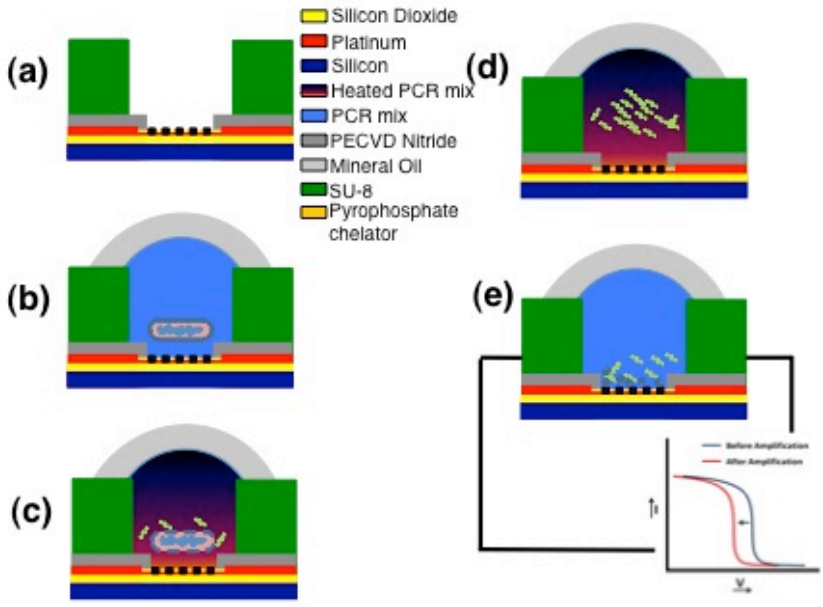

Figure 1: (a) A cross section of the proposed device. Each well will contain 3-5 transistors, each serving their own individual purpose from heating to sensing. (b) A pathogen will be flowed inside the well through microinjection or microfluidics to become the template for PCR. (c) Using AC electroporation through one of the transistors, bacteria will be lysed to release the template DNA. (d) PCR amplification will be achieved by changing the input voltage to the heater transistor. (e) The non-heating transistors inside each well will sense pyrophosphates generated via PCR in order to electrically sense amplification.

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EXPERIMENT

Our work aims to overcome the PCR-related limitations of time to result, high reagent cost, and expensive, sophisticated instrumentation through use of transistors for high-speed sub-nanoliter droplet heating on a scalable platform. To this end, we have developed a novel process to selectively heat silicon field effect transistors (FETs) using an AC voltage-mediated strategy. [3] The experimental procedure involves applying an AC voltage at 10MHz and 10-25 V_{rms} between the shorted source/drain of a transistor and the bulk substrate (Figure 2(a)). A fringing electric field directly above the targeted device causes dielectric relaxation of water ions in solution, allowing ultra-rapid heating with temperature stabilization within ~10ms. In our scheme, a droplet (<1nL) of PCR solution is placed on the device (Figure 2(b) & (c)). Changing the applied voltage allows us to control the temperature profile within the sub-nanoliter droplet. We have studied the range of temperatures possible through simulations and on-chip calibrations using the melting temperature of fluorescently labeled dsDNA FRET probes. Our results show that we are able to achieve the temperatures required for PCR and due to the rapid nature of this heating method, the time to complete 30 cycles of PCR is less than 5 minutes. Together, these characteristics help to enable a truly ultra-rapid, point-of-care test.

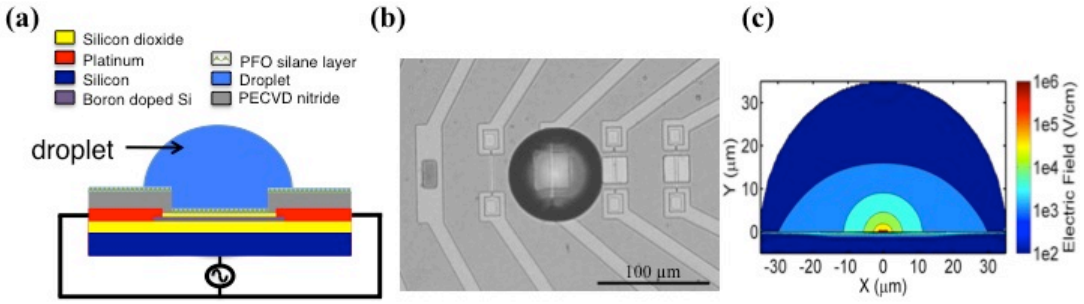

Figure 2: (a) A cross section of the device with a droplet placed over a single heating unit. (b) A bright-field image of a droplet on a transistor. The droplet's volume is approximately 100pL. (c) The fringing electric field from an applied signal of $22V_{rms}$ at 10MHz is simulated. The electric field is highly localized above the device surface.

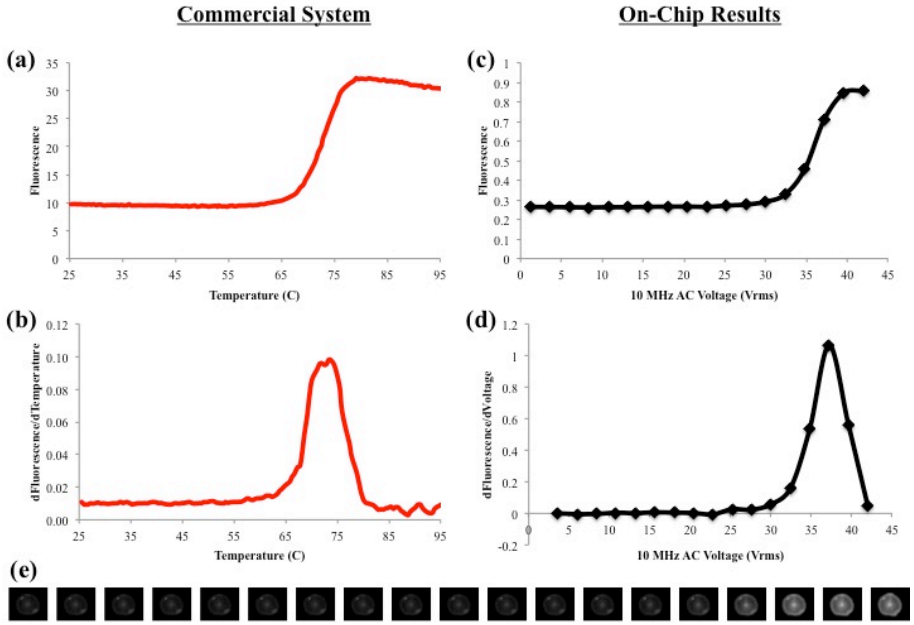

Figure 3: (a) and (b) A melting curve from a commercial real-time PCR system is shown with a 74C FRET construct. (c) and (d) A melting curve from our device is shown. Increasing the applied AC voltage increases the temperature within the droplet. By taking the derivative of the recorded fluorescence from (c), we can extract the melting voltage of the 74C FRET construct. (e) Fluorescence images of the droplet used for (c) and (d).

Additionally, we have devised a method to locally lyse individual cells on a transistor. This acts as a necessary first step to an amplification reaction as it is imperative that the DNA of the cell be released. We are able to use the same AC technique shown in Figure 2 to induce irreversible electroporation of a cell. As seen in Figure 2(c), the fringing electric field is strongly localized at the device surface and exceeds 1e6 V/cm for an applied signal of $22V_{rms}$. This strong of an electric field can be used to create a transmembrane voltage across a cellular membrane. [4] If the transmembrane voltage exceeds a certain amount ~1V, irreversible electroporation will occur and the cellular contents

will be released into the surrounding environment. [5] As shown in Figure 4, we have used this technique to perform highly localized single cell lysis. This allows for DNA release from targeted cells without the need for chemicals, mechanical forces or high temperatures that might interfere with a downstream PCR assay.

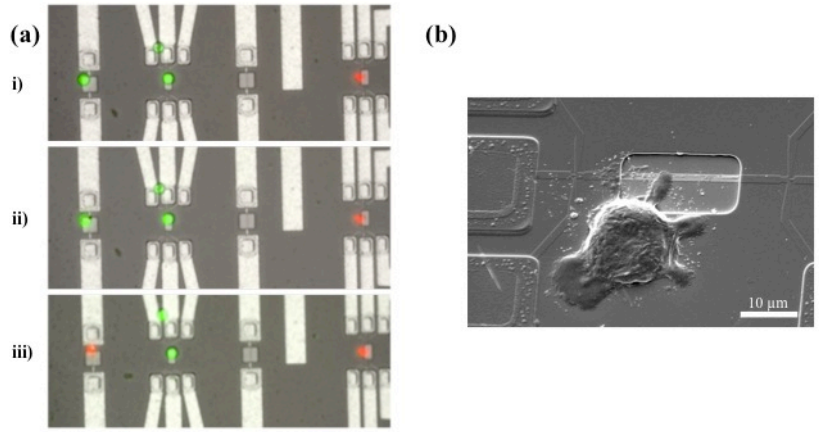

Figure 4: (a) A cell on the far left of the set of images is lysed by an applied electric field of 600mV. By (iii), the cell has taken up propidium iodide in the solution and begun to fluoresce as red. This indicates damage to the cellular membrane and death of the cell. (b) An SEM of a cell that has been lysed using the AC electroporation technique. Significant damage in the cellular membrane is evident.

In order to overcome the need for a traditional optical detection of PCR amplification, our novel heating technique on a transistor can be coupled with the biosensing capabilities of the nanowire transistor. Field effect transistors (FETs) work by sensing modulations in conductivity between the source and drain when a charged entity, like DNA, binds near the sensor surface. By functionalizing the surface of the device, highly sensitive, label-free, dynamic detection of specific chemical or biological molecules is possible. [1] Our lab has demonstrated the ability to sense hydrogen and pyrophosphate selectively on transistors. [7] [8] These ions can be used for detection of PCR amplification, as both are generated with nucleotide incorporation in the elongation phase of PCR. We are currently working to implement this technology with our previous heating and electroporation results.

By utilizing micro-fabrication techniques and semiconductor processing skills, as well as our previously demonstrated transistor-based heating technology [3], we are working to develop a novel platform capable of ultra-rapid thermocycling, highly localized cellular electroporation and the potential for electrical detection of PCR. Using our own fabricated nanowire transistors arrays, we have shown the ability to: (i) lyse individual cells, (ii) rapidly heat sub-nanoliter volumes while monitoring DNA denaturation, and (iii) electrically detect pH, pyrophosphates, and nucleic acids. With further development of our integrated system, PCR—the gold-standard of pathogen detection in the laboratory—could be made a fast, portable, inexpensive method for on-site testing of a variety of the world's most deadly infectious pathogens.

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