Disposable Silicon-based Micro-qPCR for Rapid Detection of Pathogens

Firat Güder[1]
Estefania Nunez-Bajo[1], Michael Kasimatis[1], Yasin Cotur[1], Tarek Asfour[1], Alexander Silva Pinto Collins[1], Ugur Tanriverdi[1], Max Grell[1], Matti Kaisti[1,2], Guglielmo Senesi[1], Karen Stevenson[3]
1Department of Bioengineering, Imperial College London, London SW7 2AZ, UK
2Department of Future Technologies, University of Turku, 20500 Turku, Finland
3Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh, Scotland EH26 0PZ, UK
guder@imperial.ac.uk

Rapid screening and low-cost diagnosis play a crucial role in choosing the correct course of intervention e.g., drug therapy, quarantine, no action etc. when dealing with highly infectious pathogens. This is especially important if the disease-causing agent has no effective treatment, such as the novel coronavirus SARS-CoV-2 (the pathogen causing COVID-19), and shows no or similar symptoms to other common infections. We report a disposable silicon-based integrated Point-of-Need (PoN) transducer (TriSilix) that can chemically-amplify and detect pathogen-specific sequences of nucleic acids (NA) quantitatively in real-time. [1] Unlike other silicon-based technologies, TriSilix can be produced at wafer-scale in a standard laboratory (Figure 1); we have developed a series of methodologies based on metal-assisted chemical (wet) etching, electroplating, thermal bonding and laser-cutting to enable a cleanroom-free low-cost fabrication that does not require processing in an advanced semiconductor foundry. TriSilix is, therefore, resilient to disruptions in the global supply chain as the devices can be produced anywhere in the world. To create an ultra-low-cost device, the architecture proposed exploits the intrinsic properties of silicon and integrates three modes of operation in a single chip: i) electrical (Joule) heater, ii) temperature sensor (i.e. thermistor) with a negative temperature coefficient that can provide the precise temperature of the sample solution during reaction and iii) electrochemical sensor for detecting target NA. Using TriSilix, the sample solution can be maintained at a single, specific temperature (needed for isothermal amplification of NA such as Recombinase Polymerase Amplification (RPA) or cycled between different temperatures (with a precision of ±1.3 °C) for Polymerase Chain Reaction (PCR) while the exact concentration of amplicons is measured quantitatively and in real-time electrochemically. A single 4-inch Si wafer yields 37 TriSilix chips of 10×10×0.65 mm in size and can be produced in 7 hours, costing ~US $0.35 per device. The system is operated digitally, portable and low power – capable of running up to 35 tests with a 4000 mAh battery (a typical battery capacity of a modern smartphone). We were able to quantitatively detect a 563-bp fragment (Insertion Sequence IS900) of the genomic DNA of M. avium subsp. paratuberculosis (extracted from cultured field samples) through PCR in real-time with a Limit-of-Detection of 20 fg, equivalent to a single bacterium, at the 30th cycle. Using TriSilix, we also detected the cDNA from SARS-CoV-2 (1 pg), through PCR, with high specificity against SARS-CoV (2003).

REFERENCES

[1] Ultra-low-cost integrated Silicon-based transducer for On-site, genetic detection of pathogens (BioRvix 10.1101/2020.03.23.002931)

FIGURES

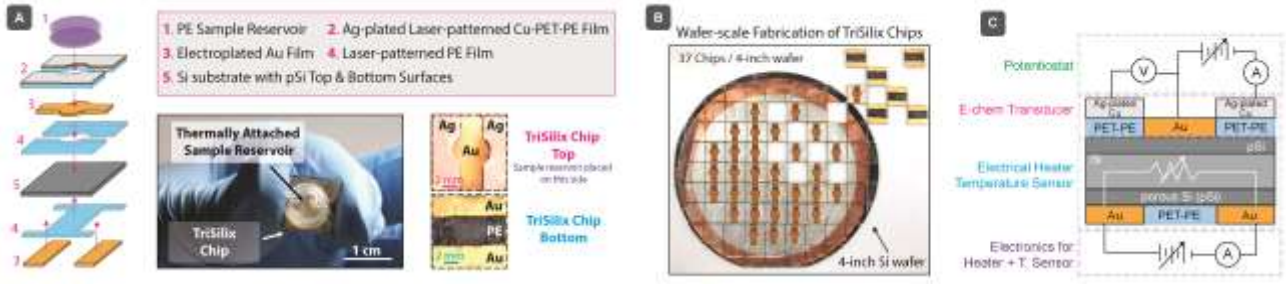

Figure 1: (A) Schematic illustration of construction of a TriSilix chip and photographs of the actual device. (B) Wafer-scale fabrication of TriSilix using a 4-inch Si wafer. Each wafer yield 37 chips. (C) Schematic illustration of the functional building blocks of TriSilix that provide trimodal operation for integrated nucleic acid amplification and detection.