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Chip based Amplification and Detection of Influenza C Virus using Dielectrophoresis

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Dielectrophoresis (DEP) is an electrokinetic phenomenon that governs the behavior of polarizable, dielectric media in an external, non-uniform electric field [1]. The phenomenon of DEP, in recent years has been exploited at microscopic scales as a droplet microfluidic (DMF) sample handling methodology and demonstrated practical utility to facilitate the electro-manipulation of minute quantities (sub µL to pL) of aqueous samples on tops of patterned surfaces [2]. Such sample manipulations include: the rapid and precision dispensing of an array of daughter droplets from a larger parent sample droplet, at specific on-chip locations [2] and the subsequent transport of the daughter droplets [2]. The DEP droplet dispensing and manipulation capabilities have been utilized for a variety of different on-chip nucleic acid detection assays, mostly using laboratory grade synthetic bio-samples.

The sample handling limitations for DEP based DMF devices often arise from surface adsorption and loss of droplet contact angle while working with enzymes and other macro-molecules [3]. This limitation in sample handling has however been overcome recently by employing DMF devices, fabricated with Nano-textured super hydrophobic surfaces [3]. These super hydrophobic DEP microfluidic devices are capable of both dispensing and subsequent manipulation of aqueous enzyme, protein and other large macro-molecule samples. The thrust of the proposed research work focuses on the development of DEP based lab-on-chip (LOC) device that integrates sample preparation, on-chip nucleic acid amplification test (NAAT) using a thermal control unit (TCU), targeted for the detection of Influenza 'C' virus.

Figure A shows the schematic of the designed microfluidic device. Figure B shows the cross-sectional layout of the DEP DMF device. The fabricated device consists of two patterned metal layers. The bottom metallization layer (200 nm Cr) is patterned to create micro-heater and RTD (Resistance Temperature Detector) sensor, labeled as the thermal control unit in Figure A. The designed micro-heater and RTD sensor is schematically shown in Figure A. The TCU layer is passivated with a 500 nm Si_3N_4 dielectric layer. The top metallization layer (200 nm Al) is patterned to create the microfluidic electrode architecture, required for the LOC device. Schematic of the designed microfluidic electrode architecture is shown in Figure A. The microfluidic electrode architecture consists of three different DMF actuation methodologies, namely: single surface Electrowetting (EW), Liquid-DEP (L-DEP) and Electrostatic droplet actuation or, Droplet-DEP (D-DEP). EW electrode architecture is used to dispense and combine, large (~ 1 µL) aliquot of cDNA sample, clinically extracted at the Provincial Health Laboratory, Calgary, AB, from patient's nasal swab samples and (~ 1µL) aliquot of NAAT master mix. The NAAT master mix used in this work was formulated specifically for the on-chip NAAT detection of Influenza 'C' virus. The PCR Droplet Cycler electrode architecture (Figure A) is then utilized to cycle the prepared PCR sample droplet over the thermal zones, achieved using the TCU. During this on-chip NAAT reaction, the PCR droplet is cycled up to 25 times and a continuous mode Photomultiplier Tube (PMT) set-up is used to extract the real time PCR amplification curve. Subsequently, the amplified sample is diluted (up to 10X) using a TRIS-EDTA dilution buffer and transferred over to the parent loading site of the post amplification screening matrix (Figure A, Figure C). The screening matrix is utilized to rapidly analyze the amplified nucleic acid content using a select set of bio-markers (synthesized Molecular Beacons: MB1 and MB2) in order to verify the presence or, absence of Influenza 'C' virus in the amplified sample. The post amplification detection assay for Influenza 'C' virus, over a 2x2 multiplexed screening matrix, was analyzed using the PMT based set-up and the results reported in micro-graphs of Figure C.

The final outcome of the research work will characterize the performance of the integrated DEP DMF technology for on-chip NAAT of Influenza virus detection in clinical patient samples in comparison to the conventional laboratory based NAAT detection.

References:
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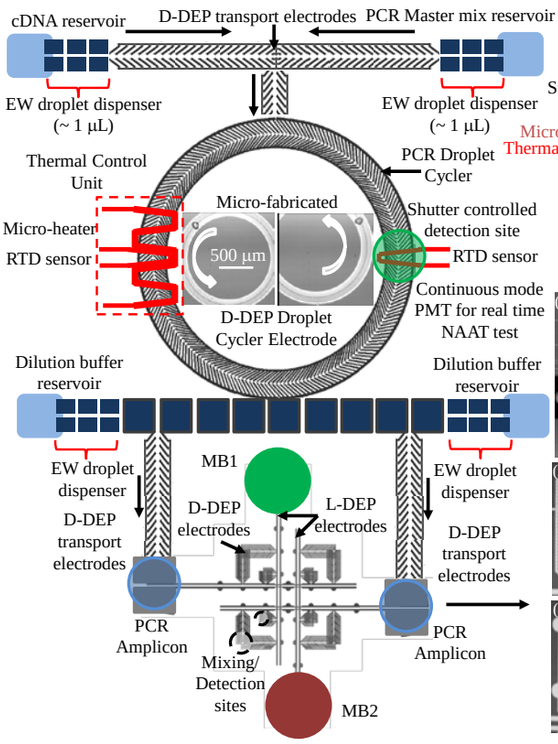
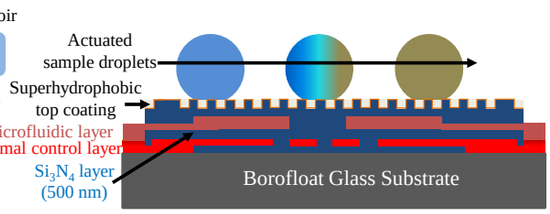
3) Prakash R., Papageorgiou D. P., Papathanasiou A. G., Kaler K. V. I. S., "Dielectrophoretic Liquid Actuation on Nano-Textured Super Hydrophobic Surfaces", *Sensors and Actuators B: Chemical*, 182, 351-361, 2013.

(A) Schematic of the integrated DMF electrode architecture



(B) Cross-Sectional view of the DEP based DMF chip



(C) Results of the post-amplification Influenza 'C' detection