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A Low-Cost Disposable PDMS Microfluidic Cartridge with Reagent Storage Silicone Blisters for Isothermal DNA Amplification

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Abstract : Over the past decade the increase of sexually transmitted infections (STIs) especially in the developing world due to high cost and lack of sufficient medical testing have given rise to the need for a rapid, low cost point of care medical diagnostic that is disposable and most significantly reproduces equivocal results achieved within centralised laboratories. This paper present the development of a disposable PDMS microfluidic cartridge incorporating blisters filled with reagents required for isothermal DNA amplification in clinical diagnostics and point-of-care testing. In view of circumventing the necessity for external complex microfluidic pumps, designing on-chip pressurised fluid reservoirs is embraced using finger actuation and blister storage. The fabrication of the blisters takes into consideration three proponents that include: material characteristics, fluid volume and structural design. Silicone rubber is the chosen material due to its good chemical stability, considerable tear resistance and moderate tension/compression strength. The case of fluid capacity and structural form go hand in hand as the reagent need for the experimental analysis determines the volume size of the blisters, whereas the structural form has to be designed to provide low compression stress when deformed for fluid expulsion. Furthermore, the top and bottom section of the blisters are embedded with miniature polar opposite magnets at a defined parallel distance. These magnets are needed to lock or restrain the blisters when fully compressed so as to prevent unneeded backflow as a result of elasticity. The integrated chip is bonded onto a large microscope glass slide (50mm x 75mm). Each part is manufactured using a 3D printed mould designed using Solidworks software. Die-casting is employed, using 3D printed moulds, to form the deformable blisters by forcing a proprietary liquid silicone rubber through the positive mould cavity. The set silicone rubber is removed from the cast and prefilled with liquid reagent and then sealed with a thin (0.3mm) burstable layer of recast silicone rubber. The main microfluidic cartridge is fabricated using classical soft lithographic techniques. The cartridge incorporates microchannel circuitry, mixing chamber, inlet port, outlet port, reaction chamber and waste chamber. Polydimethylsiloxane (PDMS, QSil 216) is mixed and degassed using a centrifuge (ratio 10:1) is then poured after the prefilled blisters are correctly positioned on the negative mould. Heat treatment of about 50C to 60C in the oven for about 3hours is needed to achieve curing. The latter chip production stage involves bonding the cured PDMS to the glass slide. A plasma coroner treater device BD20-AC (Electro-Technic Products Inc., US) is used to activate the PDMS and glass slide before they are both joined and adequately compressed together, then left in the oven over the night to ensure bonding. There are two blisters in total needed for experimentation; the first will be used as a wash buffer to remove any remaining cell debris and unbound DNA while the second will contain 100uL amplification reagents. This paper will present results of chemical cell lysis, extraction using a biopolymer paper membrane and isothermal amplification on a low-cost platform using the finger actuated blisters for reagent storage. The platform has been shown to detect 1x105 copies of Chlamydia trachomatis using Recombinase Polymerase Amplification (RPA).

Keywords : finger actuation, point of care, reagent storage, silicone blisters
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