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Total integrated slidable and valveless solid phase extraction-polymerase chain reaction-capillary electrophoresis microdevice for mini Y chromosome short tandem repeat genotyping

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ABSTRACT

A fully integrated slidable and valveless microsystem, which performs solid phase DNA extraction (SPE), micro-polymerase chain reaction (μPCR) and micro-capillary electrophoresis (μCE) coupled with a portable genetic analyser, has been developed for forensic genotyping. The use of a slidable chip, in which a 1 μL-volume of the PCR chamber was patterned at the center, does not necessitate any microvalves and tubing systems for fluidic control. The functional micro-units of SPE, μPCR, and μCE were fabricated on a single glass wafer by conventional photolithography, and the integrated microdevice consists of three layers: from top to bottom, a slidable chip, a channel wafer in which a SPE chamber, a mixing microchannel, and a CE microchannel were fabricated, and a Ti/Pt resistance temperature detector (RTD) wafer. The channel glass wafer and the RTD glass wafer were thermally bonded, and the slidable chip was placed on the designated functional unit. The entire process from the DNA extraction using whole human blood sample to identification of target Y chromosomal short tandem repeat (STR) loci was serially carried out with simply sliding the slidable chamber from one to another functional unit. Monoplex and multiplex detection of amelogenin and mini Y STR loci were successfully analysed on the integrated slidable SPE-μPCR-μCE microdevice by using 1 μL whole human blood within 60 min. The proposed advanced genetic analysis microsystem is capable of point-of-care DNA testing with sample-in-answer-out capability, more importantly, without use of complicated microvalves and microtubing systems for liquid transfer.

1. Introduction

STR typing is considered as a gold standard method for modern forensic human identification, and has been widely applied for paternity testing, missing person investigation, mass disaster victim identification, and clinical diagnosis (Jobling and Gill, 2004; Jeffreys et al., 1991; Gill et al. 1985; Bond and Hammond 2008; Gemayel et al., 2010). Current efforts in the forensic communities have been dedicated to improving the detection sensitivity, high-throughput, cost, and data reliability in the STR fingerprinting (Poon et al., 2009; Ahn et al., 1996; Walker et al., 2003; Jobling and Tyler-Smith (2003); Simpson et al., 1998). In comparison to the current state-of-art technology, the microfluidic based STR typing method holds several advantages such as low reagent consumption, fast analysis time, high potential for full integration, low contamination risk, and automation from sample input to data analysis (Choi et al., 2012a, 2012b; Hurth et al., 2010). Microfluidic devices for DNA preparation from biological samples (Reedy et al., 2010; Park et al., 2012), PCR (Lounsbury and Landers, 2013; Sun et al., 2007; Zhang and Ozdemir, 2009), and CE (Chen et al., 2010; Yeung et al., 2009; Aboud et al., 2010) have been well developed so far. In addition, partial integration (Legendre et al., 2006; Lounsbury et al., 2013; Woolley et al. 1996) and total integration (Liu et al., 2008; Reedy et al., 2011) has been demonstrated on a single device.

Hopwood et al. (2010) reported an integrated microdevice to produce multiplex forensic STR profiles from buccal swab samples. Genomic DNA from the buccal swab samples was directly delivered to the DNA processing cartridge for DNA purification and PCR amplification. The fluorescence-labeled PCR amplicons were separated in an 11-cm CE channel and detected with 1.2 bp resolution by a laser induced fluorescence detector. However, the

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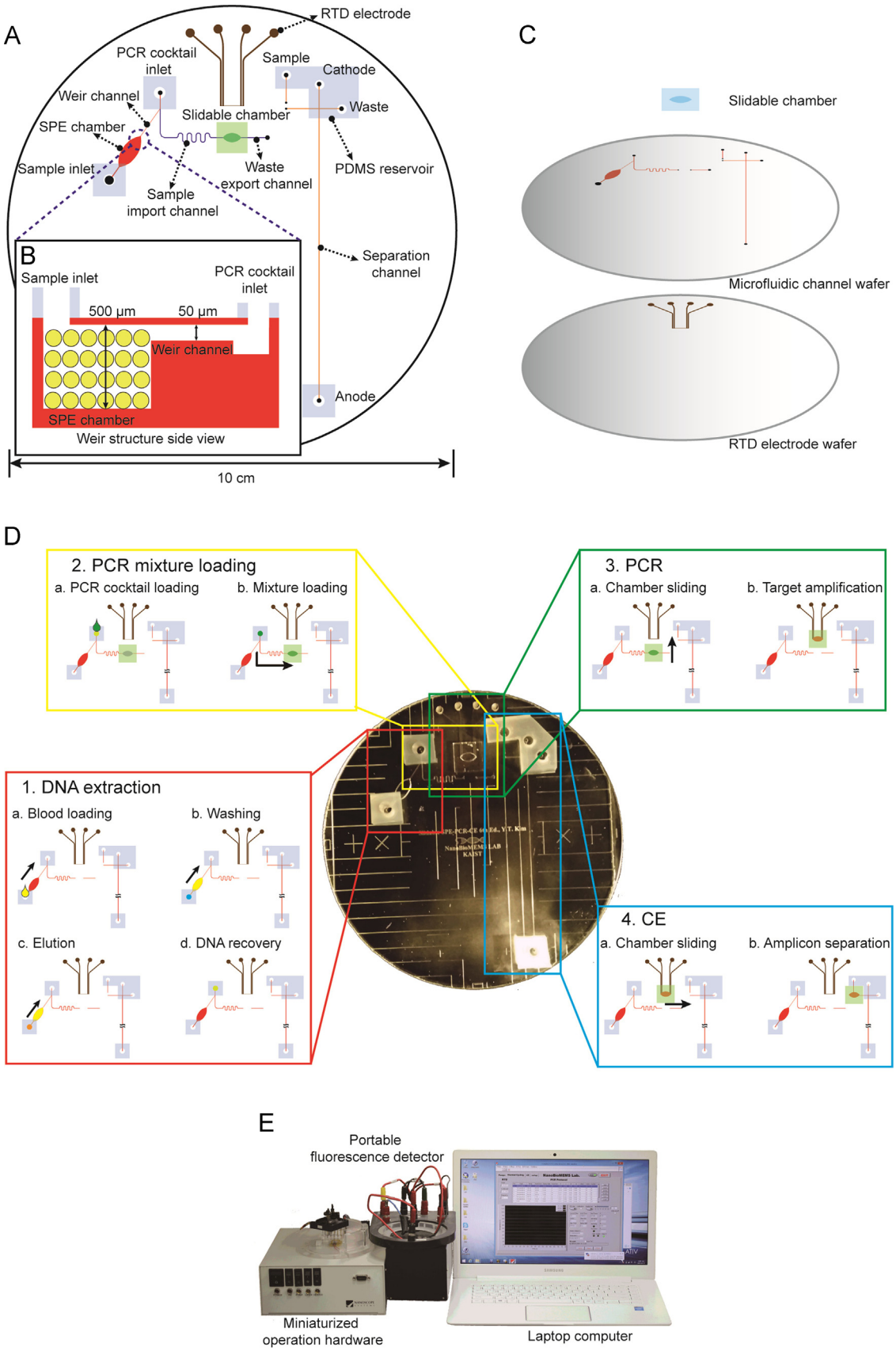

Fig. 1. (A) The structure of the slidable SPE-μPCR-μCE microdevice which consists of a SPE chamber with a weir structure, a sample import and waste export channel, a slidable chamber, RTD electrodes and micropatterned CE channels. (B) A magnification view of the SPE chamber and the weir structured microchannel. (C) Exploded view of the slidable SPE-μPCR-μCE microdevice. (D) A digital image of the slidable SPE-μPCR-μCE microdevice with operation process. (1) Solid phase DNA extraction step. Human whole blood sample loading, washing buffer injection and DNA elution were serially performed. (2) PCR mixture loading step. Purified DNA and PCR cocktail mixture was introduced to the slidable chip. (3) PCR step. The slidable chip was move to the PCR region and PCR thermal cycling was performed. (4) CE step. Followed by the target amplification, the slidable chamber slid to the CE region for the amplicon separation and detection. (E) Photograph of the portable instrumentation for operating the slidable microchip with a miniaturized operation hardware, a portable fluorescence detector, and a laptop computer.