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Micropillar array chip for integrated white blood cell isolation and PCR

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Abstract

We report the fabrication of silicon chips containing a row of 667 pillars, 10 by 20 μm in cross-section, etched to a depth of 80 μm with adjacent pillars being separated by 3.5 μm. The chips were used to separate white blood cells from whole blood in less than 2 min and for subsequent PCR of a genomic target (eNOS). Chip fluid dynamics were validated experimentally using CoventorWare™ microfluidic simulation software. The amplicon concentrations were determined using microchip capillary electrophoresis and were >40% of that observed in conventional PCR tubes for chips with and without pillars. Reproducible on-chip PCR was achieved using white blood cell preparations isolated from whole human blood pumped through the chip.

Keywords: Micropillars; Blood cell isolation; Polymerase chain reaction; Microfluidic modeling

1. Introduction

Over the past decade, successful PCR, and other biochemical reactions such as ligase chain reaction on silicon-glass and plastic-based microchips have been reported [1–5]. This technology has evolved to microfluidic chips for cell isolation, cell extraction, amplification, and analysis, although no commercial products have emerged as yet [6–10]. One component of a typical sample preparation for a genetic analysis test is blood cell isolation. Cell separation has been achieved by several methods including a fluorescence-activated cell sorter (FACS) microfabricated on a microchip [11,12] and blood cells have been sorted in a microfabricated lattice contained within a microchip [13]. On-chip dielectrophoresis has been employed to separate live *Listeria* bacteria from heat-treated *Listeria* [14], and red blood cells (RBCs) have been separated from human Jurkat cells [15]. Immunospecific isolation using a biochip in which antigen was immobilized onto a glass substrate has been effectively used in a 10 min separation of CD4-positive cells from whole blood [16]. Finally, white blood cells (WBCs) have been separated from other components of whole blood (RBCs, platelets, serum) via size exclusion using a row of micropillars or a weir inside a chamber within a microchip [10].

Previously we have used both silicon weirs and pillars for on-chip cell isolation [4]. The previous cell isolation chip based on pillars used relatively few pillars (25–120 pillars) and consequently its capacity for cell isolation was limited. In this report we describe the microfluidic simulation-aided design, optimization and fabrication of a silicon-glass microfluidic chip containing a row of 667 high-aspect ratio pillars for white blood cell isolation from whole blood. Cell isolation was followed by on-chip PCR amplification of white blood cell genomic DNA, and off-chip quantitation of the PCR products using a commercially available capillary electrophoresis microchip. We also compared the effect on a PCR reaction of the increased surface area in a pillar chip using chips with and without the array of pillars.

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2. Experimental protocol

2.1. Chip fabrication

Briefly, a 900 Å thick layer of nitride was deposited onto double side polished <100>orientation, 500 ± 20 μm thick silicon wafers (Virginia Semiconductor, Fredericksburg, VA). Using standard photolithographic techniques and reactive ion etching, pairs of 1 mm squares, 30 mm on center were patterned through the nitride masks. After removal of photoresist, squares were etched to a depth of approximately 425 μm using 25% KOH at 92 °C. After nitride removal via 48% hydrofluoric acid, a 540 nm thick oxide layer was deposited onto the wafers. Again, using standard photolithographic techniques and reactive ion etching, chip structures were patterned through the oxide mask. Chip structures were etched into the wafers to a depth of 80 μm using reactive ion etching (the Bosch process). After removal of the oxide mask via 48% hydrofluoric acid, a final 200 nm thick layer of thermal oxide was deposited onto the wafers to passivate the surface for PCR chemistry. Completed wafers were anodically bonded to 1.5 mm thick Pryex® glass (Bullen Ultrasonics, Eaton, OH) and subsequently diced. Dicing was performed at San Diego Magnetics (San Diego, CA). All other tasks were performed at the Cornell NanoScale Science and Technology Facility (Ithaca, NY). The reaction volume of each chip was 15 μL.

2.2. Microfluidic platform design

A microfluidic platform was custom-made from an acrylic block and Kel-f® valves (Fig. 1a and b). Microfluidic channels contained within the platform and valves were either 350 or 750 μm in diameter. This platform contains a recess to accommodate a thermal electric cooler (Marlow #SP1848a, Dallas, TX), and a port for nitrogen gas to be supplied beneath the TEC for heat removal. Temperature control was achieved via feedback control using a solid-state temperature sensor (Omega Engineering, Stamford, CT) glued to the thermal electric cooler, and a laser diode controller with temperature control modules (ILX Lightwave, Bozeman, MT). The controller is interfaced with a desktop computer (Dell, Round Rock, TX) via a GPIB board (National Instruments, Austin, TX). Thermocycling software was written using LabVIEW Base Development System 6.1 and PID ToolKit (National Instruments, Austin,

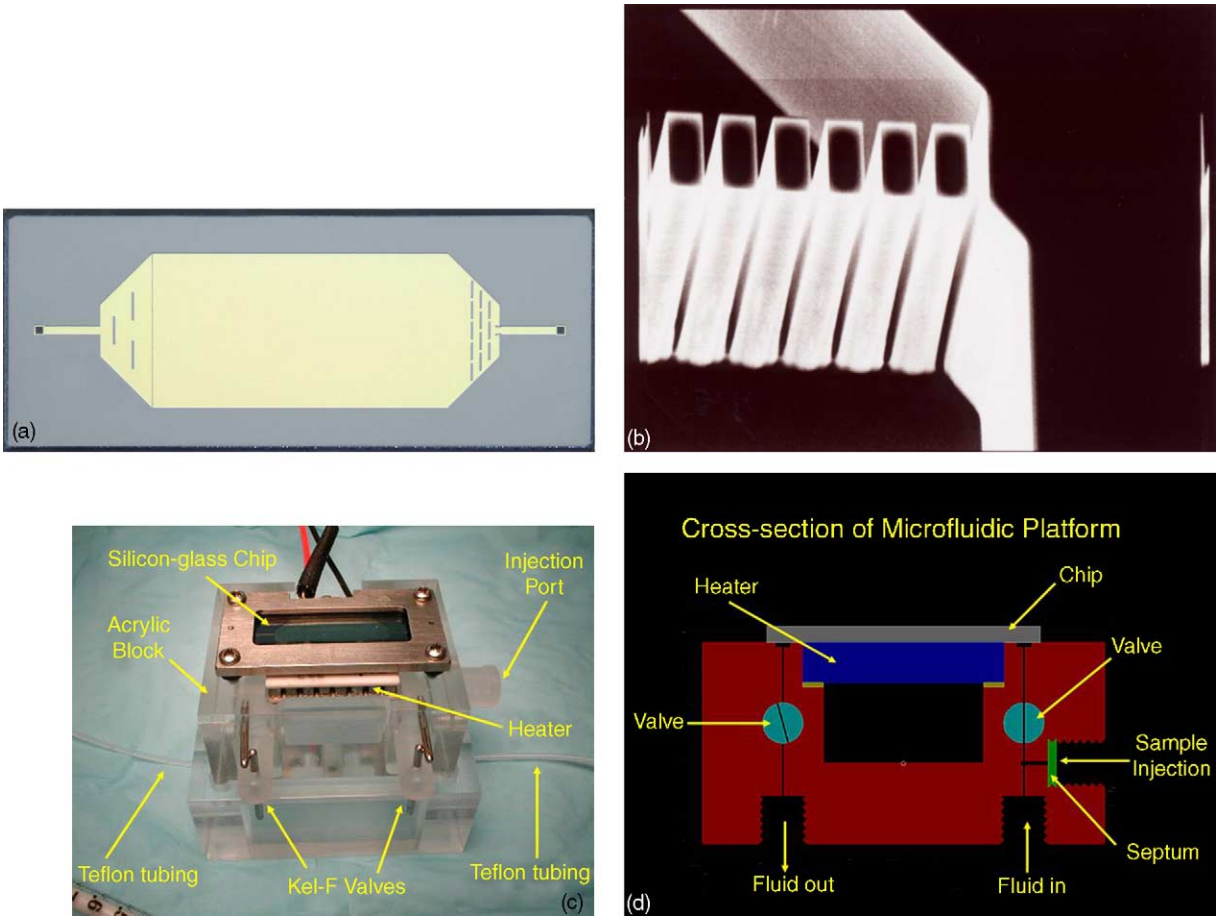

Fig. 1. Microfluidic chip and platform. (a) Photograph of chip; (b) scanning electron microscopy of part of the row of pillars; (c) photograph of microfluidic platform; and (d) cross-section of microfluidic platform depicting microfluidic channels, valves and sample injection port.