Development of a Salivary Microfluidic Diagnostic Device using Hot Embossing

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Primary CNF Tools Used: Hot press, photolithography room, ABM contact aligner, Unaxis 770 deep Si etcher, Microdrill, Objet30 3D Printer

Abstract:

Point of care diagnostic devices allow people to get fast, accurate information about their health and well-being without the need to go to a clinic or hospital. The device that we are designing will determine the concentration of cortisol from a sample of the user's saliva. Cortisol is a steroid hormone associated with stress levels and expressed in human saliva [1,2]. This microfluidic device contains a microbead-based immunoassay that we are optimizing to determine the cortisol content from a saliva sample. The device is manufactured using a hot embossing process, which uses a silicon master made with traditional lithographic processes. The device is made from a thermoplastic called Zeonor 1020R, which is a transparent, semi-rigid plastic that can be used in large-scale manufacturing processes such as injection molding and hot embossing. Nearly all the fabrication of the device is being done in the Cornell NanoScale Facility.

Summary of Research:

The microfluidic device is made using a hot embossing process, which involves the high-temperature pressing of a mold into a piece of thermoplastic. The mold that we use in our process is made of silicon and is fabricated using photolithographic processes. The design for the mold is made using L-Edit and transferred to a photomask using the Heidelberg mask writer (DWL2000). This mask is then used to transfer a pattern to a photoresist on a silicon wafer. The photoresist (SPR-220-7.0) is spun onto a bare silicon wafer, which has been previously primed in the YES vapor prime oven, to a thickness of approximately 7 µm. After spinning, the photoresist is soft baked on a 115°C hot plate for 2 minutes and 30 seconds. The wafer is allowed to sit for an hour and then exposed using the mask and the ABM contact aligner. The wafer is again allowed to sit for an hour and then is developed using the Hamatech Steag wafer processor. The pattern is now developed and can be used to etch the silicon wafer.

We etched the wafer using the Unaxis 770 deep Si etcher to a depth of 50 µm. We monitored the etch depth and etch rate using the P10 profilometer. Upon reaching the desired depth, we removed the photoresist in the chemical strip bath. We then used the Unaxis 770 again to deposit a thin layer of fluoropolymer onto the wafer in order to prevent sticking in the hot emboss process. Our masters were then ready to be used in the hot emboss process.

The hot emboss process uses the CRC Prepreg Mini Test Press, which applies heat and even pressure. The silicon master is adhered to a glass backing, for strength, and then the plastic piece is placed on top of the master, with another glass piece on top of that. This whole stack is placed in the hot press once the hot press reaches the desired temperature and pressed for several minutes. The setup is allowed to cool below the glass transition temperature of the plastic and then the pressure is released and the plastic is de-embossed. The pattern is transferred from the master to the plastic. We then drill through-holes in a blank piece of plastic using the custom-made micro drill. In our own lab, we perform a photografting procedure to increase hydrophilicity of the Zeonor surfaces and improve bonding. This blank piece is then thermally bonded to the patterned piece to create the microfluidic device in the hot press. Our microfluidic device is now complete and ready to be turned into an immunoassay. An example can be seen in Figure 1(a).

We can now flow differently sized beads into the device to create areas for antibody-antigen-fluorophore interaction. The channels after the beads are successfully

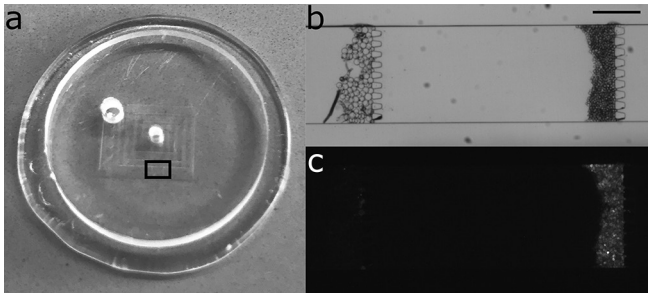

Figure 1: Microfluidic chip. a) Completed microfluidic chip with inlet at center and outlet on the left. Box depicts pillar/bead zone. b) Microscope image of channels, pillars, and different sized bead zones c) Microscope image of fluorescence on beads after flowing of AlexaFluor488 through chip.

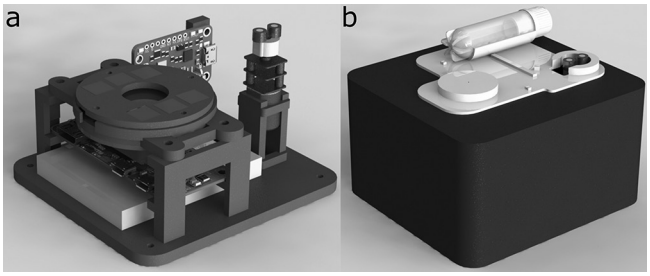

Figure 2: Rendering of 3D-printed imaging and pumping device. a) Interior assembly including Raspberry Pi Zero W, optical components, geared motor, custom peristaltic pump head, lithium-ion battery, and Powerboost 1000C. b) Exterior of device with disposable cassette attached via snap fit.

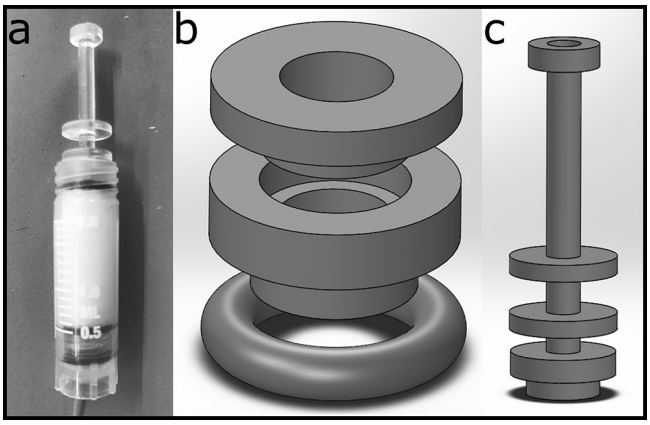

Figure 3: Saliva filtration setup. a.) Image of complete filtration setup. b.) Custom filter holder with two pieces that hold a circular filter between them and an O-ring beneath. c.) Plunger to compress saliva swab and push saliva through filter.

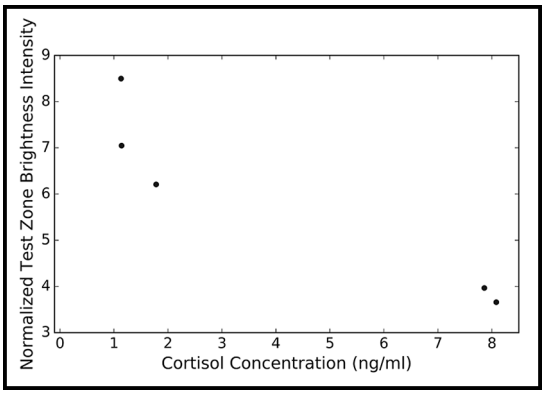

Figure 4: Preliminary fresh filtered saliva results. n=1.

added can be seen in Figure 1(b). The differently-spaced pillars allow two zones with beads with different antibodies to be separated by size. We then flow fluid with cortisol and AlexaFluor488-labeled antibodies through the device using a custom microfluidic peristaltic pump and measure the brightness of the fluorescence at the bead zones with a microscope or with our portable imaging and pumping device. A microscope image of the two bead zones with attached fluorophores can be seen in Figure 1(c). The portable imaging device is a Raspberry Pi Zero W with a camera attached, fluorescent optics, a lithium-ion battery, LEDs, and a custom peristaltic pump setup, all assembled in a 3D-printed light-tight case. This case is printed using the Objet30 Pro 3D printer and can be seen with all parts assembled in Figure 2.

Saliva samples were collected from human participants with approval from Cornell's Institutional Review Board, and then stored at 4°C until analysis. These samples were analyzed using a commercial ELISA kit to determine the concentration of cortisol in each. These samples were also filtered using a custom 3D-printed filter setup (Figure 3) and then flowed through the microfluidic chips and imaged. Some preliminary results from some samples can be seen in Figure 4.

References:

[1] Kirschbaum C, Hellhammer DH Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology, 1994 - Elsevier.

[2] Umeda T, Hiramatsu R, Iwaoka T, et al. Use of saliva for monitoring unbound free cortisol levels in serum. Clin Chim Acta 110:245-253. doi: 10.1016/0009-8981(81)90353-3 (1981).