Rapid Point of Care Diagnostic for Sepsis Stratification

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Primary CNF Tools Used: ABM contact aligner, Class II resist room, Heidelberg 2000 mask writer, profilometer, PDMS casting station, Hamatech wafer developer, UNAXIS 770 deep Si etcher, resist stripper, MVD 100

Abstract:

Sepsis is a rapidly progressing, life threatening immune response triggered by infection that affects many populations worldwide. Therefore, this research aims to create a microfluidic immune cell capture device that is capable of diagnosing sepsis at the point of care. To do this, we created a mold on a silicone wafer using deep silicone etching, which we then cast in polydimethylsiloxane (PDMS) and adhered to a glass slide to create a functional device. A drop of blood is lysed and quenched to remove red blood cells before leukocytes are captured on antibody coated pillars, where they can be quantified and correlated to a sepsis diagnosis. Development of an accurate point of care diagnostic device for sepsis would improve diagnostic speed, reduce hospital costs, and save many lives.

Summary of Research:

Sepsis is a life-threatening condition caused by the body's drastic response to microbial infection, which triggers a cascade of events that can lead to organ failure and death [1]. Neutrophil CD64 expression has been shown to increase under septic conditions making it a potential biomarker for sepsis [2,3]. In response to bacterial infection, neutrophil CD64 has been shown to be upregulated as early as one hour after infection [4]. This project aims to create a microfluidic device that can measure changes in quantities of immune cells and correlate them with a diagnosis of either onset, early stage, or late stage sepsis within hours from a whole blood sample. The long-term goal is to be able to quickly, inexpensively, and reliably determine the on-set and current stage of sepsis at the point of care so that treatment can be properly administered.

The microfluidic device will consist of two main sections: a lysing/quenching region and an antibody chamber for cell capture (Figure 1). Approximately 10 uL of whole blood will be exposed to lysing buffer followed by quenching buffer over a length of small pipes. This will remove red blood cells in order to obtain pure leukocyte populations. CD4+ T cells, CD4+CD25+ T reg cells, and CD64+ neutrophils will be captured on antibody coated pillars in the antibody capture chamber. These cells will be measured by counters or fluorescence and analyzed by a smart phone.

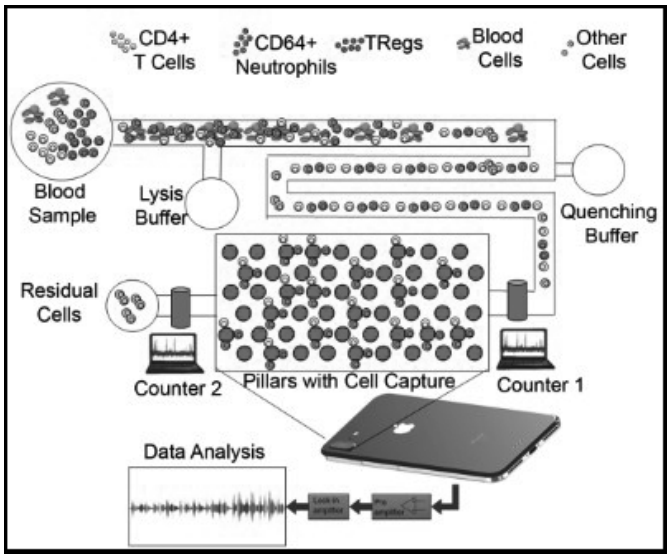
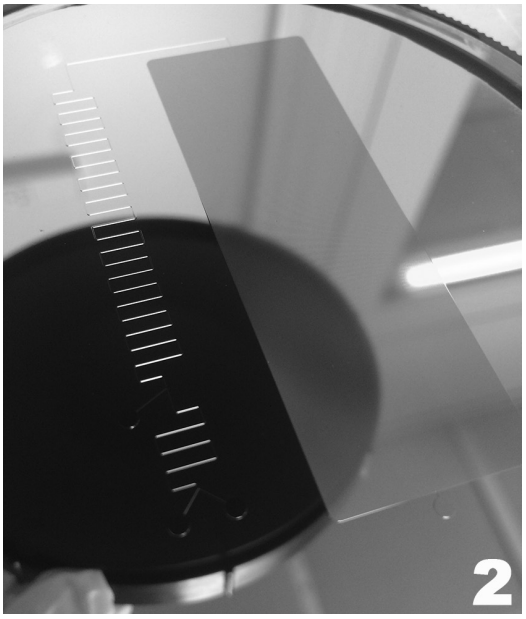


Figure 1: Schematic of microfluidic chip design.



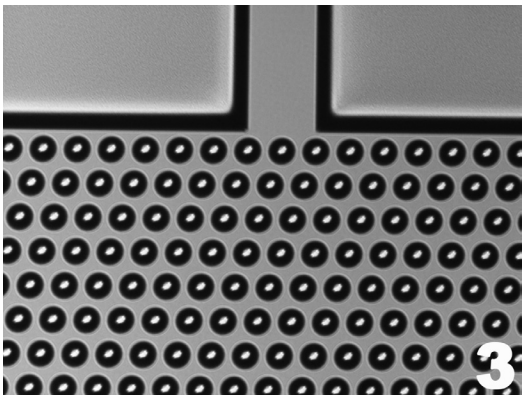


Figure 2, top: *Deep etched silicone wafer mold.* ***Figure 3, middle:*** *Ten times magnification of silicone mold inlet to antibody capture chamber.* ***Figure 4, bottom:*** *PDMS device cast from the silicon mold.*

The Cornell NanoScale Facility was used to create a silicon mold of the antibody chamber and the lysing/quenching region (Figure 2). The antibody capture chamber is approximately 20 mm by 60 mm with ~ 200,000 pillars that are 40 μm in diameter and 60 μm tall (Figure 3).

The mold was first attempted using SU-8 photolithography, but the large area of pillars had trouble staying adhered to the silicon wafer. As an alternative, the UNAXIS 770 deep silicon etcher was used. We were able to achieve uniform pillars much faster and more reliably using this method.

The final device is cast in polydimethylsiloxane (PDMS) and adhered to a glass slide using plasma cleaning (Figure 4). Prior to casting, we coated the silicon wafer mold in (1H,1H,2H,2H-perfluorooctyl)trichlorosilane (FOTS) using the MVD 100 tool to help prevent the PDMS from sticking to the mold.

The next steps are to test the device for functionality and ability to reliable capture and quantify cells.

References:

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