Microfluidic Chip Manufacturing for Point of Care Sepsis Diagnosis

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Primary CNF Tools Used: ABM contact aligner, Hamatech wafer developer, UNAXIS 770 deep silicon etcher, Anatech resist strip, PDMS casting station

Abstract:

Sepsis is a life-threatening condition affecting the immune system that causes the body to damage its own organs. The current diagnostic standard, consisting of monitoring organ dysfunction with a cell culture, is ineffective as it can take days, while sepsis can cause death within hours. The goal of the project is to develop a point of care microchip that can provide an accurate diagnosis from a drop of blood in 30 minutes or less by measuring the level of expression of white blood cell antigens associated with sepsis. The device utilizes a lysing and quenching process to destroy red blood cells while preserving white blood cells, and then captures white blood cells containing target antigens on antibody-coated pillars. Research has been focused on optimizing the lysing and quenching process, with a secondary goal of redesigning the pillar arrangement to maximize cell capture. In on-bench experiments, we have been able to achieve nearly 100% red blood cell lysis while maintaining less than 10% white blood cell lysis. Initial testing of cell capture on the pillars has resulted in a new design containing less pillars to increase the ease of imaging, manufacturing, and flow.

Summary of Research:

Introduction. Contributing to over 250,000 deaths per year in the US alone, sepsis alters the body's immune response to infection, leading to organ damage and immunosuppression [1]. The current diagnostic standard for sepsis includes a cell culture and a scoring system known as the Sequential Organ Failure Assessment (SOFA), which involves measuring a collection of non-specific clinical symptoms for levels of organ dysfunction [2]. This current method of diagnosis is ineffective, partly due to its lack of specificity, and because it can take days to obtain results, whereas the survival rate for sepsis decreases by 7.7% each hour that administration of antimicrobials is delayed [3]. The objective of this research is to create a microchip that can provide an accurate septic or non-septic diagnosis within 30 minutes using a drop of whole blood.

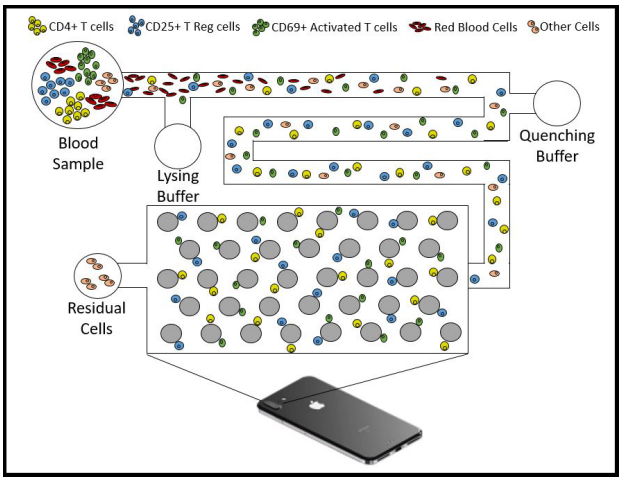

Figure 1: A simplified representation of the processing of blood and capturing of specific white blood cells on pillars in the microchip.

Design and Fabrication. The design of the microchip contains S-shaped channels and a section of antibody-coated pillars (Figure 1). When blood enters the channels, it mixes with a lysing buffer, which lyses the

red blood cells allowing for easier passage of white blood cells through the device. After the blood and lysing buffer have mixed for a few seconds, a quenching buffer is added to halt the lysing process before the white blood cells are damaged. The white blood cells enter the pillar section, and cells containing the target antigens corresponding to the pillar antibodies adhere to the pillars. The use of fluorescent antibodies allows for fluorescence smartphone imaging of the captured cells. The measured fluorescence corresponds to the level of expression of the target antigens, correlating to the presence of sepsis. To manufacture the device, first a mold is made from deep etching a silicon wafer. The mold is then cast in polydimethylsiloxane (PDMS) and plasma bonded to a glass slide. A pump system is attached via tubing to move fluid through the device.

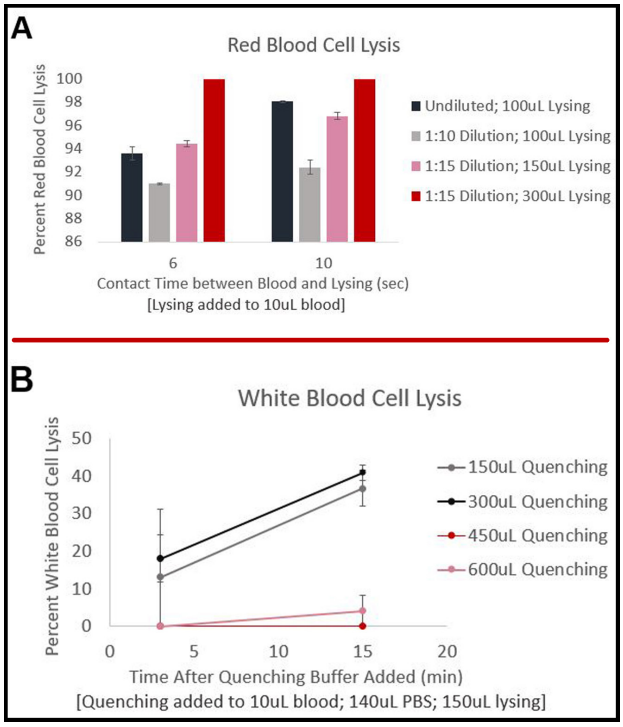

Figure 2: A) Percentage of red blood cell lysis at differing blood to lysing volume ratios for undiluted and diluted blood. B) Percentage of white blood cell lysis over time for differing quenching volumes after quenching buffer is added to diluted blood and lysing buffer.

Results and Conclusions:

A lysing buffer consisting of formic acid and saponin and a quenching buffer with sodium carbonate and phosphate buffered saline (PBS) were selected [4]. We tested various ratios of lysing buffer and quenching buffer on the bench to determine the most effective proportions to lyse the red blood cells, while limiting the damage to white blood cells. As Figure 2A shows, we were able to achieve 100% red blood cell lysis using 10 µL of blood diluted 1:15 in PBS and a lysing volume twice as large as the volume of diluted blood. We determined that the quenching volume must

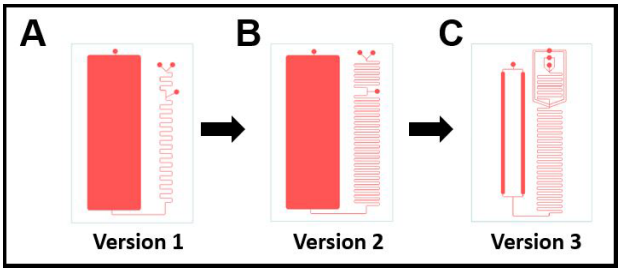

Figure 3: CAD drawings of the three versions of microchip designs.

be at least three times that of the lysing volume to achieve less than 10% white blood cell lysis over time (Figure 2B). Based on our results, we redesigned the device with longer channels to increase contact time between the blood and buffers (Figure 3B). The third version of the chip contains two channels and four channels extending from the lysing inlet and quenching inlet respectively to ensure that sufficient amounts of the buffers mix with the blood (Figure 3C). From a preliminary test of cell capture on the pillars, we determined that the large number of pillars was inhibiting fluid flow through the chip. Version 3 contains only 30,000 pillars, compared to the 400,000 pillars in the previous versions, and the pillar chamber is broken into two sections to allow for easier fluid flow and imaging of the device. From an initial on-chip lysing test using Version 3, we obtained nearly 99.5% red blood cell lysis with a flow rate of 10 µL per minute, however further testing is needed to determine the accuracy of these results.

Future Work:

Further research includes testing of the on-chip lysing and quenching process using Version 3 devices, as well as optimizing and imaging the capture of CD3+ T cells, CD4+ helper T cells, CD25+ T regulatory cells, and CD69+ activated T cells on the chip. Future work also includes incorporating a low-cost, effective pumping system to replace the current 3-syringe pump system.

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