Optimization of White Cell Enrichment from Small Blood Volumes

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UCDAVIS COLLEGE OF ENGINEERING

Magnophil

Background

White blood cells are essential for the study of immunology due to their role in the immune system. For these cells to be studied, they must be isolated from whole blood. Some of the common methods that isolate cells include centrifugation, filtration, and magnetic bead marking.

Problem

Among the common methods of white blood cell isolation, there is a lack of processes that use small amounts of blood. Most methods require an amount of blood that can only be obtained through venous blood draw, which can lead to complications such as pain, bruising, and hematoma at the venipuncture site. These side effects will harm the donor, hindering his or her desire to continue donating blood, which will make it difficult for the researcher to continue the research study.

Objective

The objective of this project is to develop a scaled-down method that will isolate specific cell types from a small amount of blood, which can be obtained through a simple finger prick, avoiding the need for venous blood draw.

Design

Magnetic Bead Isolation: This device will utilize an enrichment cocktail to isolate neutrophils from whole blood. Antibody complexes will attach to undesired cells, so that when they are mixed with a solution of dextran coated magnetic beads, the beads will attach to the undesired cells. A magnetic gradient can then be applied to separate the undesired cells from the desired cells.

Design Solution: To be able to scale down the magnetic bead isolation protocol, our team has designed a microfluidic device. The microfluidic chip consists of three inlet channels that converge into one main channel. The different inlets will be loaded with different contents: 1. Empty PBS buffer 2. Blood mixed with antibodies 3. Magnetic beads. Due to the small features of the channel, the streams will remain in laminar flow, so they will never mix. The contents of the streams will only mix in the presence of a magnet. The magnet will pull the magnetic beads from their original stream, into the adjacent stream to mix with and attach to the cells, and then will bring the undesired cells into the empty buffer stream for disposal.

Design Considerations

Laminar Flow: To ensure laminar flow, the fluid must have a low Reynold's number (Re), which is the ratio between the inertial and viscous forces of the fluid. This is dependent on the fluid viscosity, its velocity, and the dimensions of the channel.

$$Re = \frac{vW\rho}{\mu}$$

Magnetic Bead Trajectory: The channel must also be able to accommodate the movement of the beads. The beads must be able to move through each channel, without moving too quickly, to maximize the mixing time with the cells. The velocity of the beads can be described by the ratio of the magnetic field and viscous drag force.

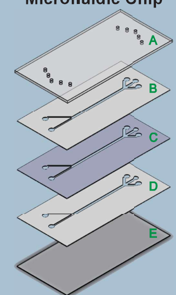
$$v = \frac{XV(\nabla \cdot B)B/\mu_0}{6\pi\eta R}$$

Fabrication

Microfluidic Chip: The chip was made by attaching double sided acrylic adhesive to both sides of a sheet of PET film. The channel features were laser cut into this sandwich and sealed on the top and bottom by glass. The top is stuck to a glass micro slide with holes that have been laser cut for the inlets and outlets. The bottom is attached to a plain glass coverslip.

Housing: The housing of the device, which secures the microfluidic chip in place and inserts the fluid and contents into microchannel, was made using a polyjet 3D printer. Soft rubber gaskets are printed directly onto the rigid body of the housing and are used to form a tight no leakage seal around the inlets and outlets of the microfluidic chip when pressed against it.

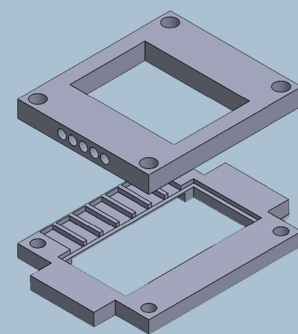
Microfluidic Chip



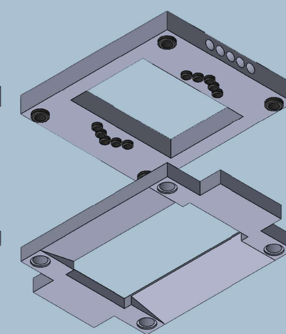
A. Glass slide with holes
B. Laser cut acrylic adhesive
C. Laser cut PET film
D. Laser cut acrylic adhesive
E. Glass coverslip

Micrfluidic Chip (Assembled)

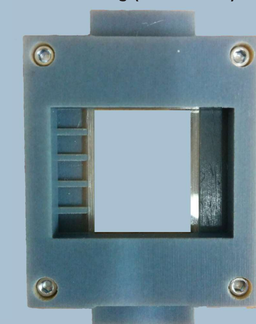
Housing



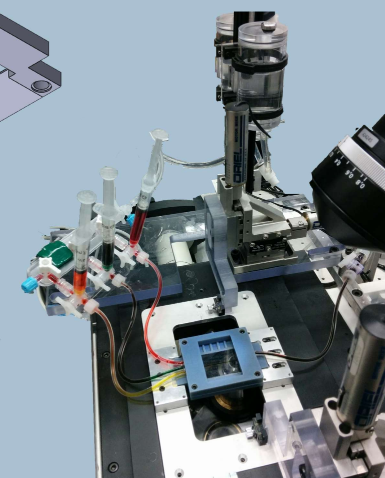
Housing (Flipped)



Housing (Assembled)



Device Installed on Microscope

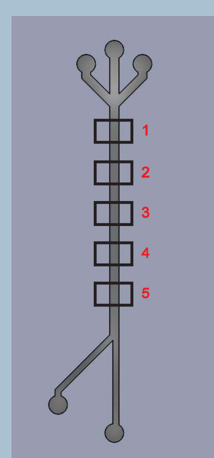


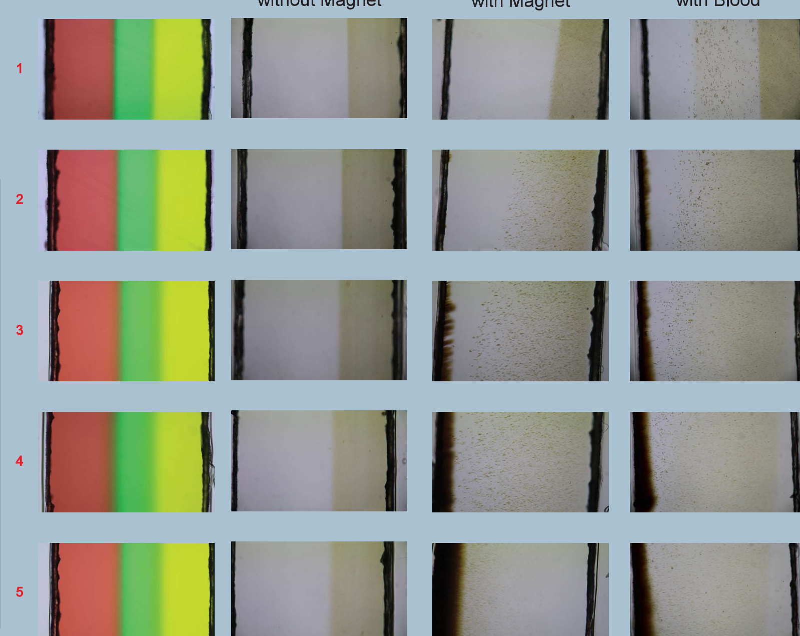
Results

Materials

- Microfluidic chip
- Housing
- Microscope (10x)
- Magnetic Beads
- Enrichment Cocktail
- Neodymium Magnet

Microchannel





Conclusions

- Food color shows that the streams will never mix, due to laminar flow
- With no magnet present, the magnetic beads will never leave their stream
- A magnet can precisely control the magnetic beads to move in their desired trajectory
- The beads can mix, bind, and move the undesired cells to the outlet channel

Acknowledgements

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