Non-instrumented CD4 Cell Separation through use of Stimuli-Responsive Reagents
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Abstract: The high antiviral drug resistance of the HIV has led to the progression of many drug-resistant mutations from the wild type virus. Prior to patient treatment, genotypic or phenotypic assays are commonly used for the detection of drug resistance. The time, cost, and complexity required for DNA extraction and to run these assays are several challenges for use in global health. A potential solution is the incorporation of Stimuli-Responsive Reagents to isolate CD4 cells, with the Oligonucleotide Ligation Assay (OLA), developed by Beck and Frenkel (2007) to detect point mutations in the HIV-1 pol genome. CD4 cell isolation can be achieved by utilizing stimuli-responsive reagents, which have been shown to facilitate effective protein and virus separations. Preliminary experiments using CD4 T cells (PM-1) and non CD4 macrophage cells (RAW 264.7) as capture targets demonstrated successful separation in RPMI-1640 and Fetal Bovine Serum (FBS), but also non-specific cell separation.

Materials and Methods

- CD4 T-Cells (PM-1) and non CD4 macrophage Cells (Raw 264.7)
- Magnetic-/Temperature-Responsive Nanoparticles (mNPs)
- Stimuli-responsive anti-CD4 polymer conjugates
- RPMI-1640, DMEM, and Fetal Bovine Serum (FBS) to culture cells
- Standard cell counting procedure with Hemocytometer used to quantify cells
- Mixed reagents, applied heat and magnetic field, and counted cell capture and supernatant

Results

- Magnetic nanoparticles separate CD4 T cells up to 68% and 93% in PBS and RPMI-1640 and FBS, respectively
- Less magnetic nanoparticles might be capturing less CD4 non-specifically
- Future Work: Isolate CD4 Cells utilizing stimuli-responsive reagents from blood samples

Conclusion

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