Fabrication and Fluid Dynamic Properties of Polymeric Particles with Varying Physical Properties*

Chris Yu,
Undergraduate Student
Department of Materials Science and Engineering
University of Michigan

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1 Abstract

The fabrication of reproducible polymeric particles with differing shapes, sizes, and porosities is a point of interest in the field of microfluidics, especially in capturing and purifying rare circulating tumor cells. Such cells are extremely difficult to capture and their study is important in the understanding of the ever changing disease of cancer and in finding a cure for it. When run through a microfluidic device, differing physical properties can cause particles to separate into different channels of a microfluidic device, a process known as particle sorting. By functionalizing such particles, their surface and physical properties can be used together to attach to different cell types found in a patient's blood and thereby help to separate the cells of interest, in this case, the circulating tumor cells. In this investigation, the synthesis and separation of particles consisting of varying physical properties is analyzed. A proof of concept study on the attachment of particles to tumor cells is also shown.

2 Introduction

Circulating tumor cells, also known as CTC's (Fig. 1), are tumor cells approximately 20 micrometers in diameter that are often found in the blood of patients with cancer and are believed to be the cause of many cancer related deaths [1]. Having separated from a primary tumor, CTC's circulate in the bloodstream and aid in the additional growth of tumors in other vital organs. This process is known as metastasis [2]

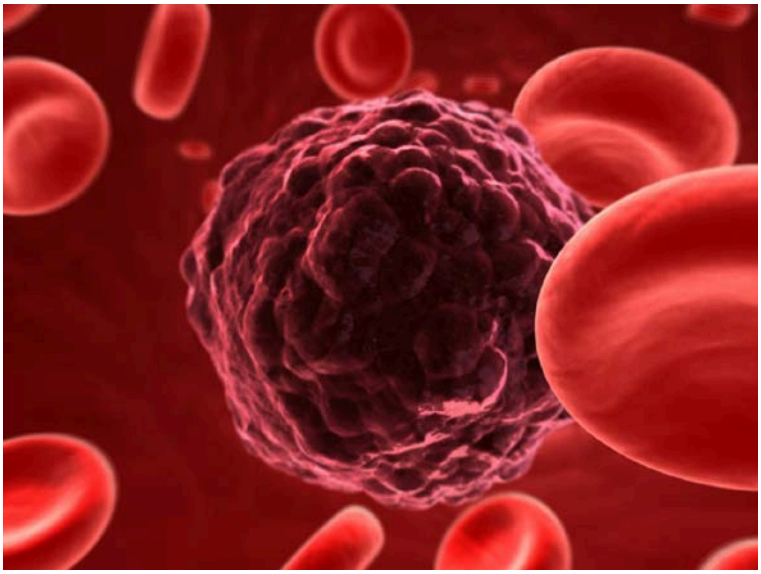


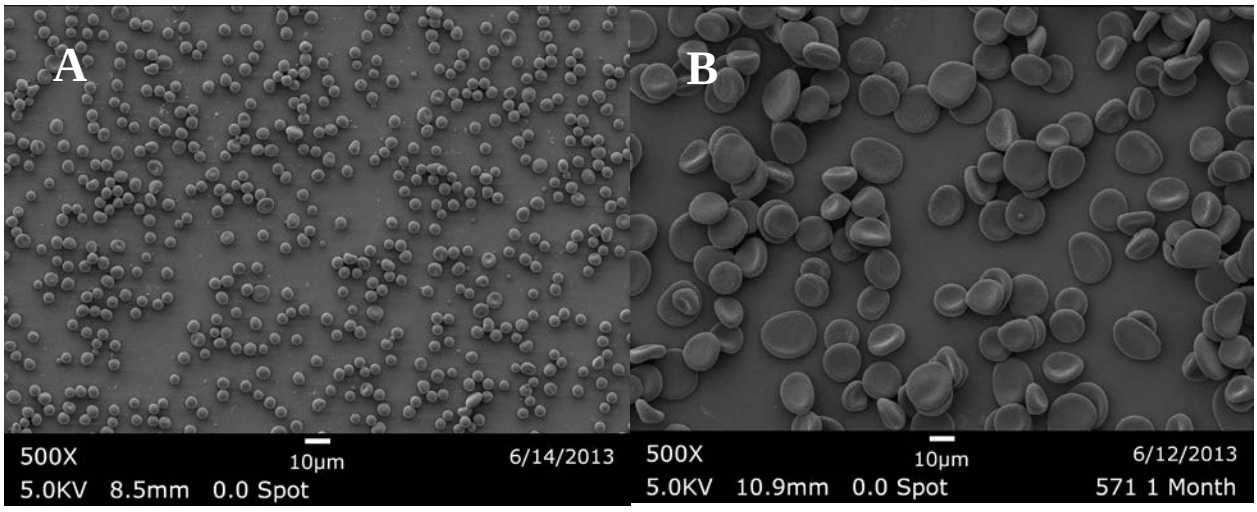
Fig. 1 Image of circulating tumor cell surrounded by red blood cells [3]

It is extremely beneficial to be able to separate CTCs from the blood circulation. By separating several CTC's from a blood sample they can be analyzed individually for a patient. The CTC's can be grown outside the body and treated with various drugs to characterize specific treatments for different types of cancer. This noninvasive method is explored using microfluidics. The concentration of CTC's in blood differs between patients and also between different types of

cancer but it is known that the concentration of CTC's is very low compared to other blood cells [1]. When using microfluidics, the rarity of CTC's makes it difficult to specifically isolate them without the contamination of other cells in the final sample [1]. As a result, there is a need in the cancer therapy field for the isolation and purification of CTC's. Herein, we propose the use of polymeric particles with unique flow dynamics to address this challenge.

Fig. 2 Microfluidic device with four different outlets. Solutions with cells and particles are run through the device and separate due to differing flow dynamics.

In order to achieve such a goal, we first investigated the separation of different particles using a microfluidic device (Fig. 2) at varying flow rates. We surface modify these particles, which allows them to attach to targeted cells [3]. Particles with attached cells will then separate into different channels based on size, shape, and porosity (Fig. 3).



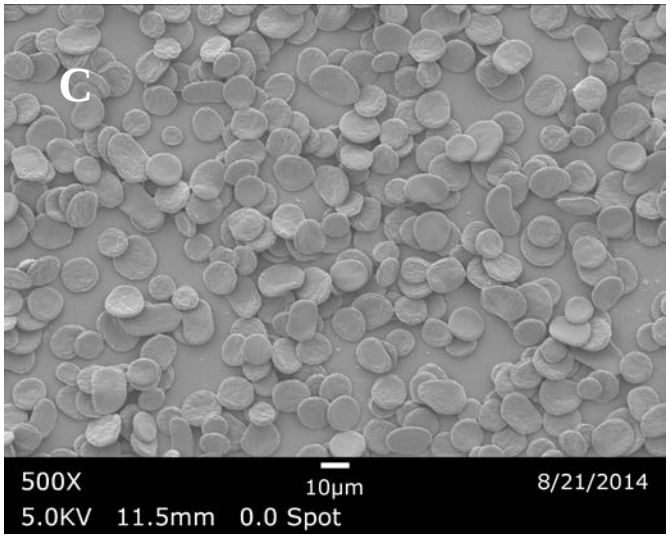


Fig. 3 Images taken using scanning electron microscopy (SEM) at 500x zoom showing particles consisting of different sizes and shapes. Image A shows spherical particles, image B shows red blood cell like particles, and image C shows particles shaped like flat disks.

Here, we have used the electrohydrodynamic co-jetting process to create particles of different sizes, shapes, and porosities and have used them to target the disease or virus in question. In this case, CTCs are targeted. The primary polymer used in creating particles is poly(lactic-co-glycolic acid) (PLGA) due to its biodegradable and non-toxic properties in the body [5]. The primary focus of this investigation is the fabrication of particles with differing physical properties, their separation in a microfluidic device, their surface modification, and their attachment to tumor cells.

2.0 Experimental Procedure

2.1 Electrohydrodynamic Co-Jetting

Electrohydrodynamic co-jetting (EHD co-jetting) is the process used to create different particles [6]. Through this process syringes are attached to a pump where the rate at which the polymeric solutions is flown can be chosen. The solutions run through joint metal needles that are attached to an electric current. As the solutions charge they create a spray of polymeric droplets that quickly evaporate to give particles. The result is biphasic (with two compartments if jetting with two syringes) particles or triphasic particles (with three compartments if jetting with three syringes) (Fig 4).

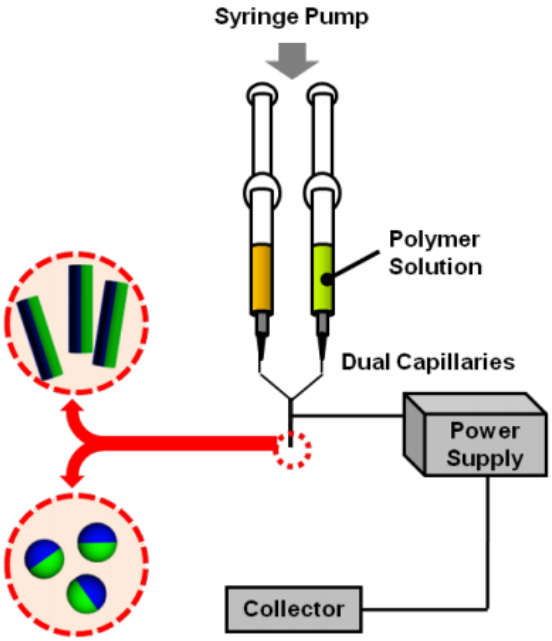


Fig. 4 Polymer solutions are drawn into syringes and pumped at different flow rates. Different flow rates will yield different sized particles. Solutions come together using two needles held together using a transparent plastic tube. The needles are connected to an electrical supply and grounded to a collector plate. Various particles and fibers can be created this using this process.

2.2 Porous vs. Non-Porous Particles

These particles were fabricated consisting of 4.5% w/v Acetal Dextran and 2.5% w/v poly (lactide-co-glycolide) acid and a 97:3 ratio of chloroform ($CHCl_3$) and dimethylformamide (DMF). These were jetted at a rate of 0.4 mL/hr. A sample of these particles was incubated at 37 C for 10 hours in a pH 5 buffer to make them porous. The acetal dextran polymer is a pH responsive polymer and releases from the particles at the lower pH, thereby creating the pores within [3].

2.3 Red Blood Cell like Particles vs. Flat Disk Particles

Red blood cell like particles (RBC's) were created using a 7.0% w/v PLGA 85:15 solution dissolved in a 97:3 ratio of $CHCl_3$:DMF using a green 2.5 mg/mL fluorescent dye. Flat disk particles were jetted using a 4.0% w/v PLGA 85:15 solution with the same ratio of $CHCl_3$ and DMF using a 3 mg/mL blue fluorescent dye. Both of these solutions were used in the EDH co-jetting process at flow rates of 1.0 mL/hr and 0.6 mL/hr for the RBC's and flat disks, respectively.

3.0 Results/Discussion

3.1 Porous vs. Non-Porous Particles

Microfluidic testing was done for porous and non-porous particles (Fig. 5).

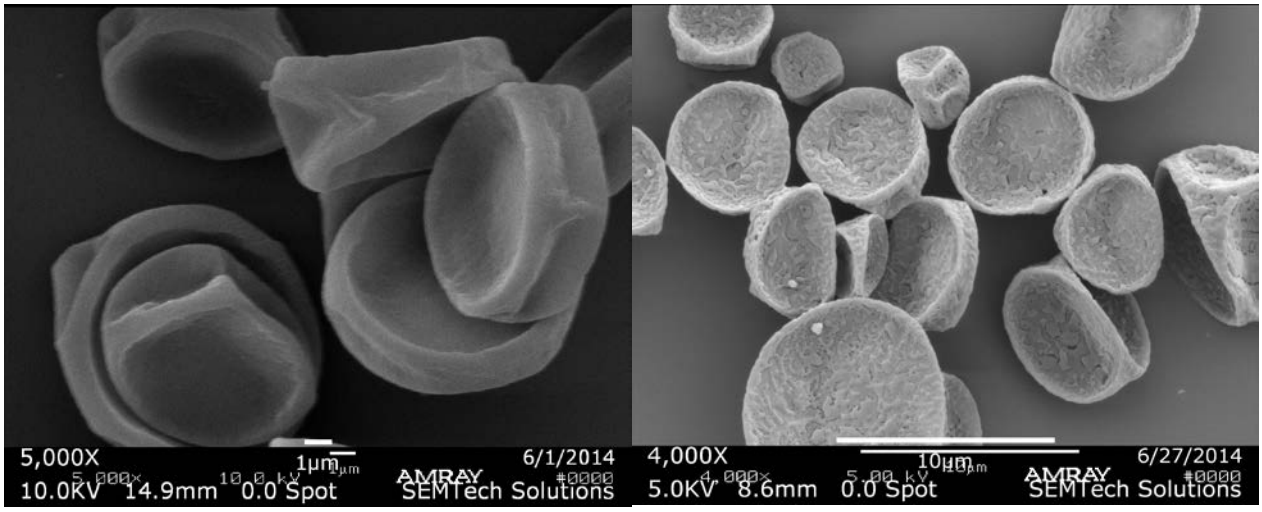


Fig. 5 The image on the left shows dextran particles immediately after jetting. Particles taken from the same jetting session are then immersed in a pH 5 buffer and incubated for 10 hours. The result is shown in the right image.

In a four channel outlet, the porous and non-porous particles did not separate into different channels. The graph below shows the percentage ratio of porous and non-porous particles separated into each outlet (Fig. 6).

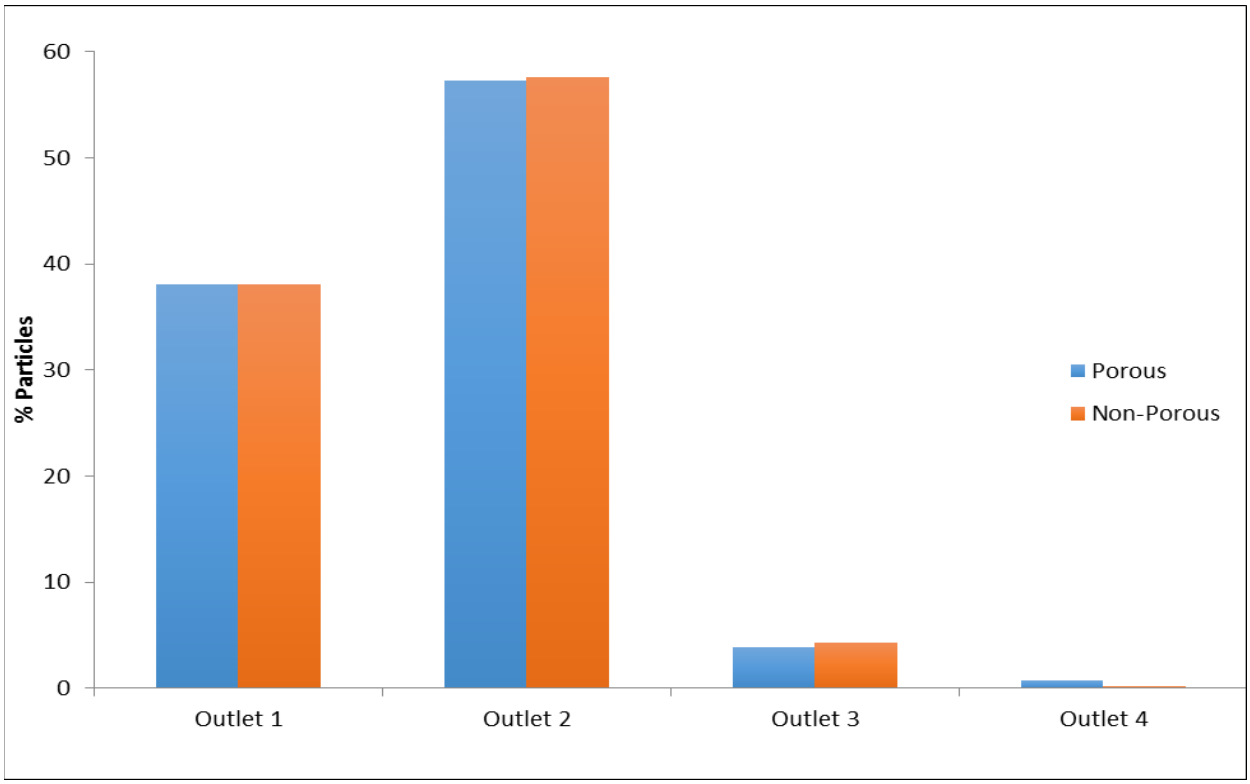


Fig. 6 Testing in a microfluidic device showed that porous and non-porous particles did not separate differently. The graph above shows that the percent of porous and non-porous particles measured in each outlet was almost identical.

The particles were collected in a phosphate buffered solution (PBS) with 1% w/v Tween 20 and passed through a 40 micron and 20 micron filter. In order to make the particle samples more consistent in size and shape, a separation method was completed using centrifugation. The steps were as follows,

1) Centrifuge at 4000 RPM for 2 minutes and dispose of the supernatant
2) Centrifuge at 300 RPM for 1 minute and dispose of the supernatant
3) Centrifuge at 2000 RPM for 2 minutes and collect pellet

3.2 Red Blood Cell like Particles vs. Flat Disk Particles

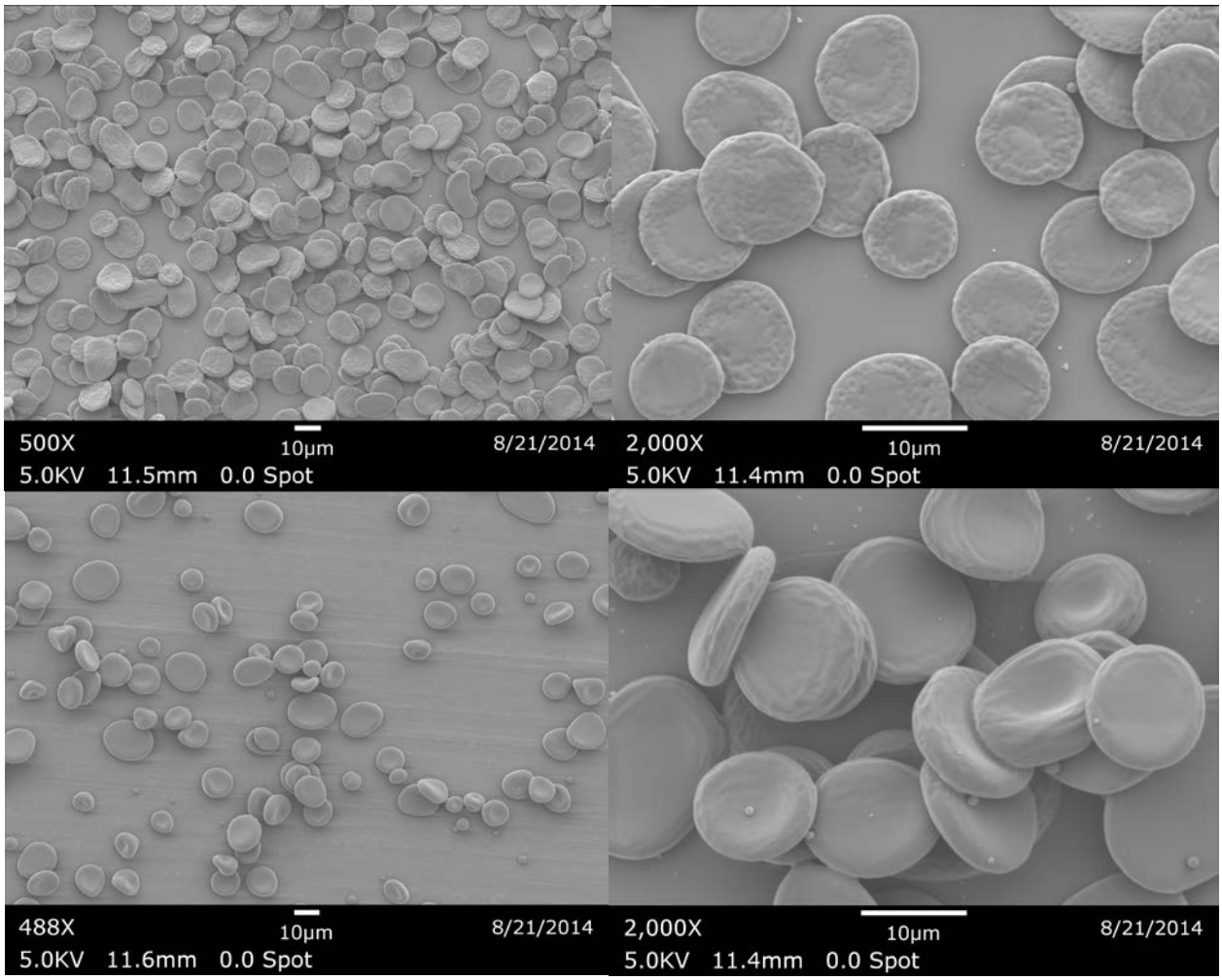


Fig. 7 The top images show flat disk particles at two different magnifications while the bottom images show red blood cell particles. It is observed that the flat disk particles are slightly wrinkled. The red blood cell like particles have a spherical bi-concave disk shape similar to red blood cell particles found in blood.

Similarly, the RBC's and flat disk particles were also collected using the same phosphate buffered solution (PBS) with 1% w/v Tween 20 and passed through a 40 micron and 20 micron

filter. In order to make the particle samples more consistent in size and shape, a separation method was completed using centrifugation. The steps were as follows,

1) Centrifuge at 4000 RPM for 1 minute (2x) and dispose of the supernatant
2) Centrifuge at 500 RPM for 1 minute and dispose of the pellet
3) Centrifuge at 2000 RPM for 2 minutes and collect all particles

The first step was done in order to separate particles smaller than 5 micron, the second step was to separate particles larger than 15 micron, and the final step was done to gather all the particles into a pellet to be collected. The pellet was then resuspended in 10 mL of PBS + 1% w/v Tween 20 solution and given to the Nagrath group to be tested in a microfluidic device. Initial results showed that RBC's and flat disk particles separated into different channels where RBCss flowed into the first outlet and flat disk particles separated into both the first and second outlet (Fig. 8).

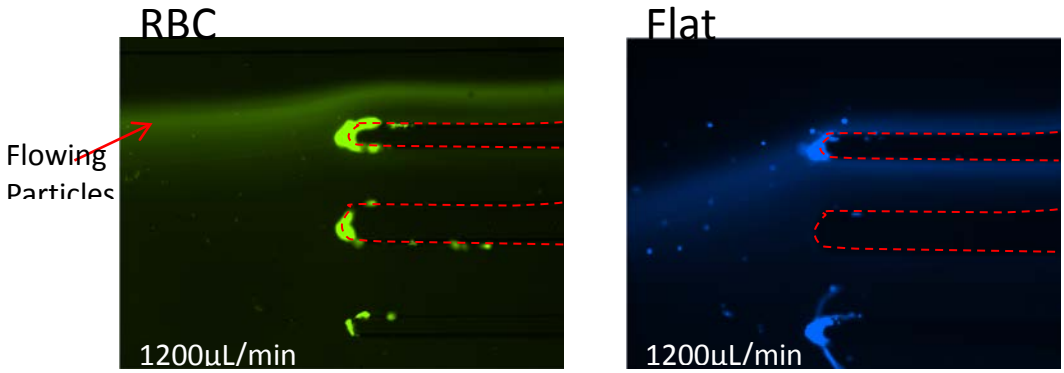


Fig. 8 Initial testing in separate microfluidic devices showed that red blood cell and flat disk particles separated into different channels. The images show that the RBCs flowed into the first outlet while flat disks flowed mostly into the second outlet. The channels are outlined in red.

The initial test was done in separate microfluidic devices but further testing in the same device showed that the two sets of particles did not separate differently (Fig. 9 and Fig. 10)

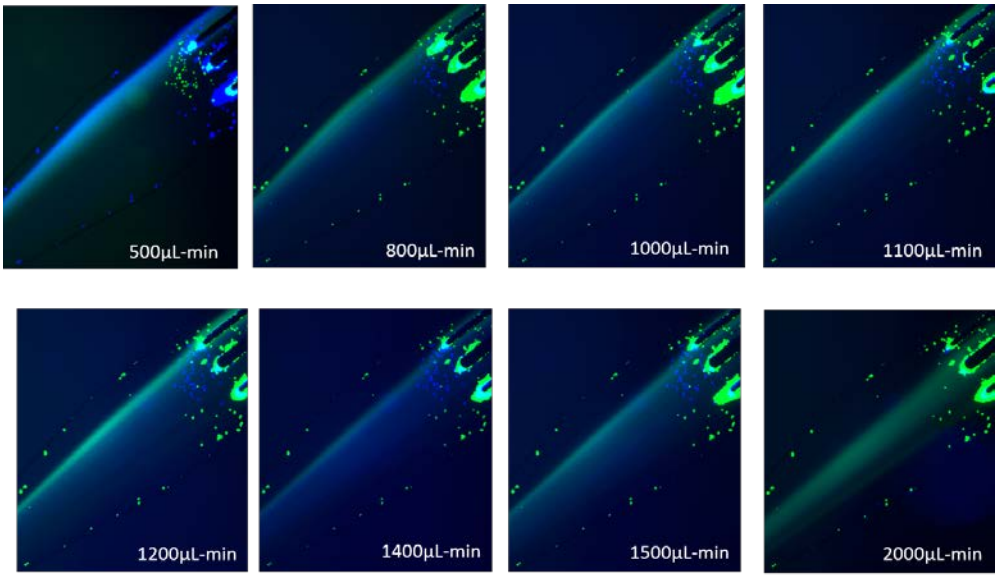


Fig. 9 Red blood cell like particles (green) and flat disks (blue) were run in the same microfluidic device at the same time at differing flow rates. These results showed that there is no separation between the sets of particles.

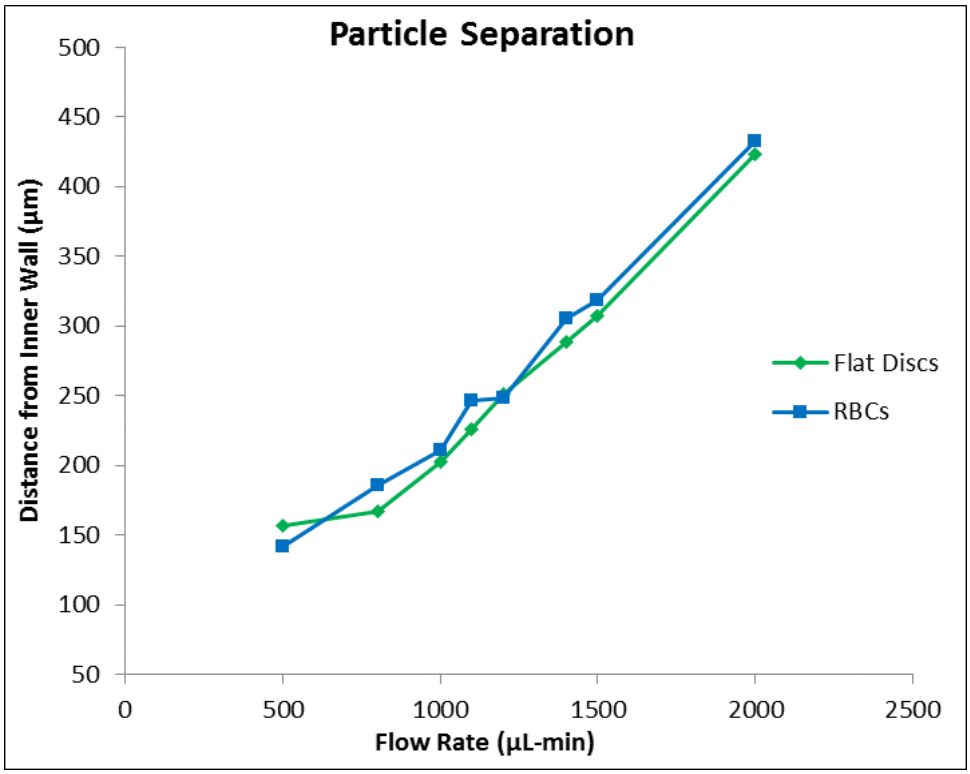


Fig. 10 Further data from the microfluidic device showing that the two particle types do not separate differently.

Testing of porous and non-porous, and RBC's and flat disks showed that these two sets flow into different outlets (Fig. 11). As such, one set of particles per group will be used for further testing.

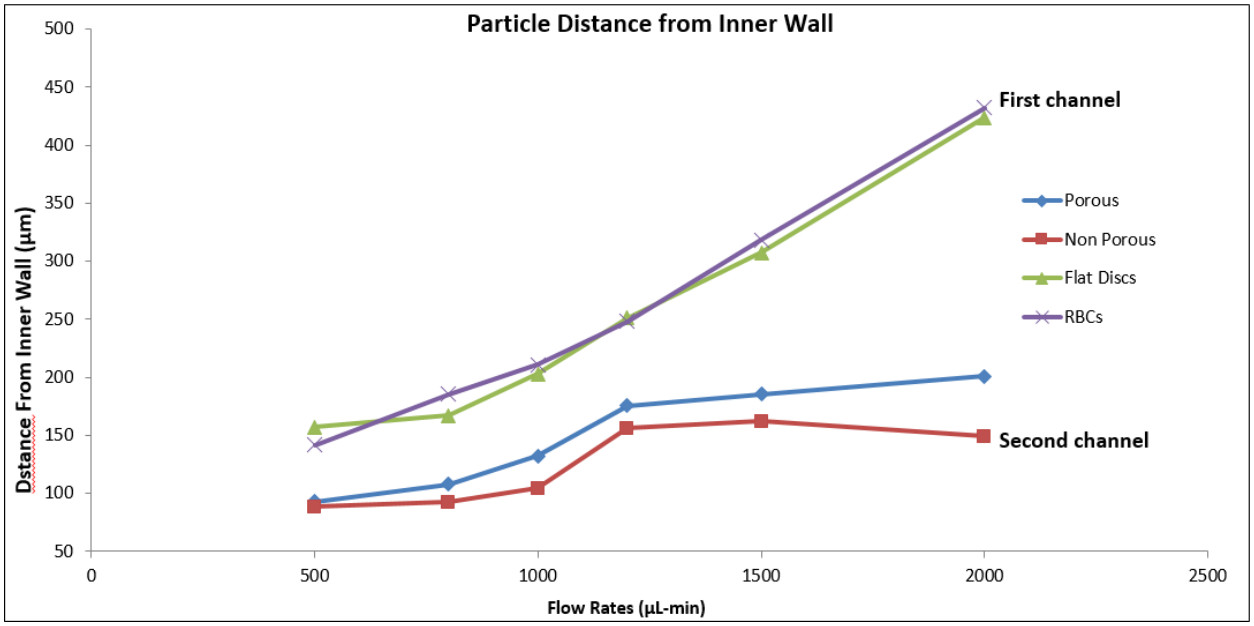


Fig. 11 Data collected from the microfluidic device shows that porous and non-porous particles flowed into the second channel while flat disks and RBC's flowed into the first channel.

3.3 Attachment of Tumor Cells

A preliminary test was done using non porous particles to confirm the ability for tumor cells to attach to particles (Fig. 12).

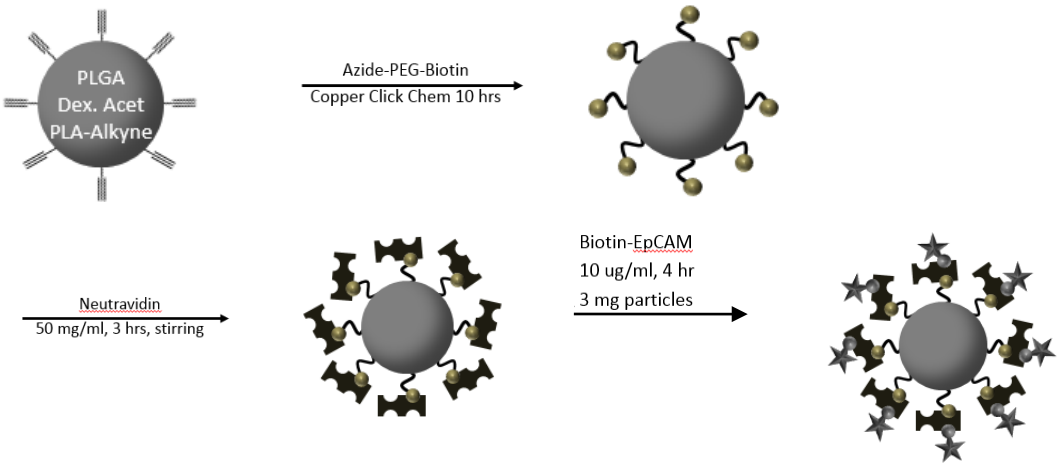


Fig. 12 Click chemistry was used to attach biotin-EpCAM to particles. The EpCAM modified particles were used in experimentation to attach cancer cells.

The results of the experiment showed that tumor cells attached to the particles (Fig. 13 and Fig. 14). These tests were done in a test tube.

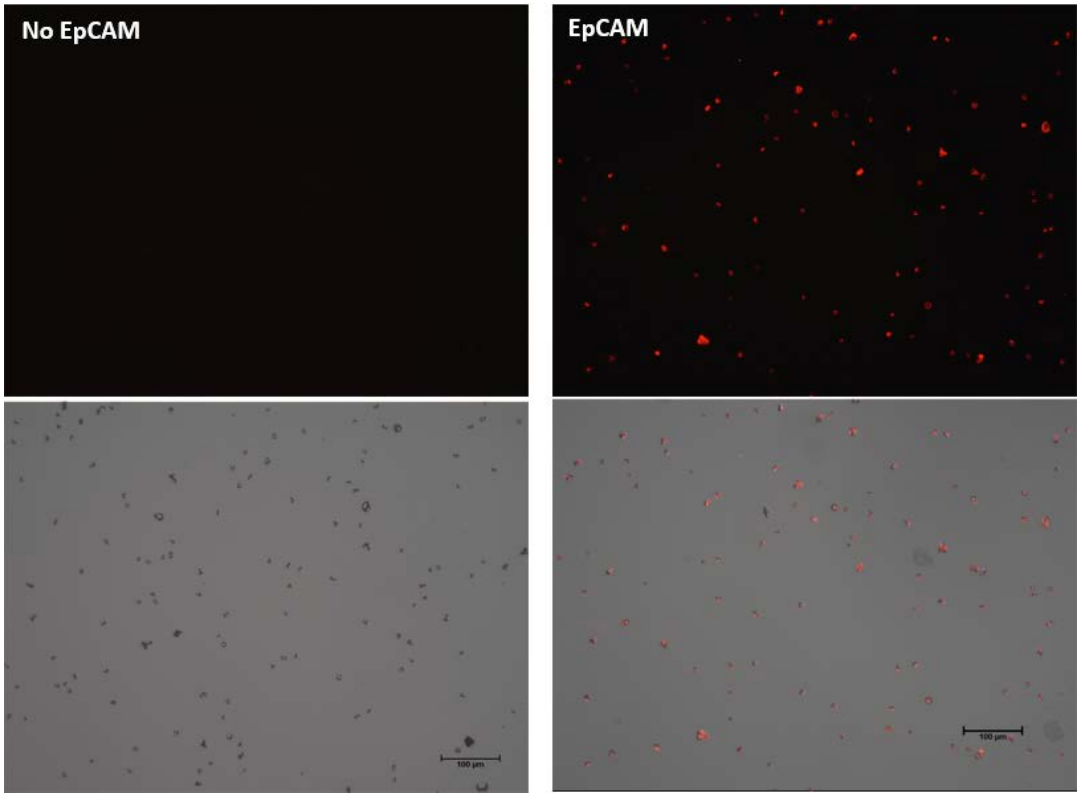


Fig. 13 Florescent images show that the EpCAM successfully attached to the particles. The images on right show, in red, the EpCAM attached particles.

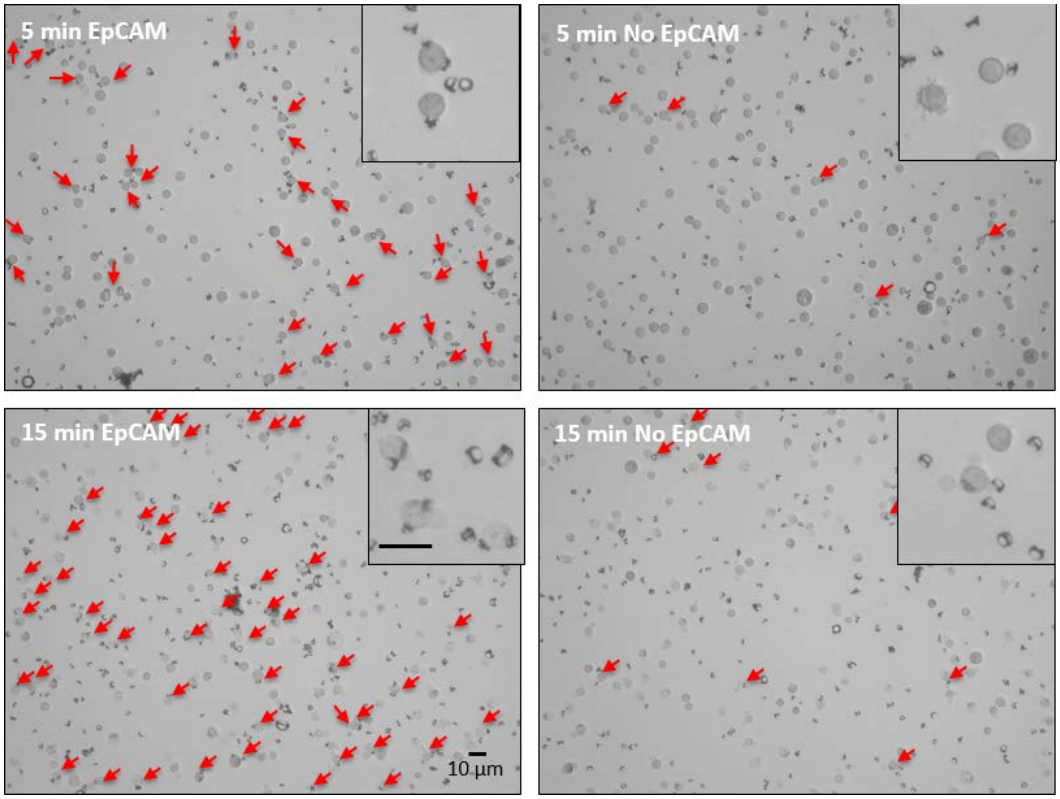


Fig. 14 After surface modification of particles, preliminary tests show that tumor cells attached themselves to the modified particles. Particles were left in solution for 5 and 15 minutes. Red arrows show the cells that have attached to particles.

4.0 Conclusion/Future Work

Experiments with polymer particles with differing physical properties have shown that flat disk particles and RBC's do not separate differently in a microfluidic device. Similarly, porous and non-porous particles do not separate into different channels either. Data showed that flat disk particles and RBC's separate into different outlets compared to porous and non-porous where RBC's and flat disks separate into the first outlet and the latter separating into the second outlet in a microfluidic device. We conclude that we potentially have different particles with different flow dynamics. Future work for this project includes further experimentation with several different formulations to create particles with other shapes which include spheres and cylinders. These particles will also be run through a microfluidic device to determine a difference in flow dynamics. When a consistent flow difference is observed between particles, they will be modified to attach to CTC's and flowed through a microfluidic device. The ultimate goal of this project is to better separate and isolate CTC's from patient blood samples for further therapy studies.

5.0 References

[1] Vasudha Murlidhar, Mina Zeinali, Svetlana Grabauskiene, Mostafa Ghannad-Rezaie, Max S. Wicha, Diane M. Simeone, Nithya Ramnath, Rishindra M. Reddy, and Sunitha Nagrath "A Radial Flow Microfluidic Device for Ultra-High-Throughput Affinity-Based Isolation of Circulating Tumor Cells." *Small* (2014).

[2] Goubran, Hadi A., et al. "Regulation of tumor growth and metastasis: the role of tumor microenvironment." *Cancer Growth and Metastasis* (2014): 9+. *Academic OneFile*. Web. 1 Sept. 2014.

[3] Bhaskar, S., Roh, K., Jiang, X., Baker, G., Lahann, J. "Spatioselective Modification of Bicompartmental Polymer Particles and Fibers Via Huisgen 1,3-Dipolar Cycloaddition" *Macromolecular Journals*

[4] Ciruclating tumor cells. (n.d.). *Circulating tumor cells.* Retrieved August 10, 2014, from http://www.pnas.org/content/110/13/4861

[5] Lee, Kyung-Bok, Kuk Ro Yoon, Seong Ihl Woo, and Insung S. Choi. "Surface modification of poly(glycolic acid) (PGA) for biomedical applications" *Journal of Pharmaceutical Sciences* Volume 92, Issue 5, Article first published online: 27 FEB 2003."

[6] Roh, K., Martin, D., Lahann, J. "Biphasic Janus particles with nanoscale anisotropy" *Nature Materials* **4**, 759 - 763 (2005)

[7] S. Rahmani, T.-H. Park, A. F. Dishman, and J. Lahann, "Multimodal delivery of irinotecan from microparticles with two distinct compartments", *Journal of Controlled Release*, (2013) 172 (1), 239–241.