Binding Selectivity of Micro-droplet Emulsions to the P-Selectin Receptor

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ABSTRACT

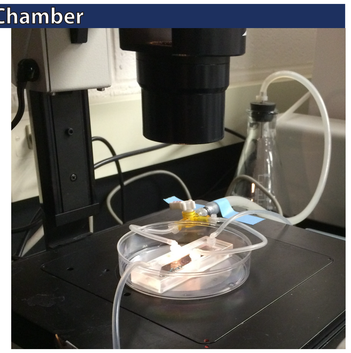
Crohn's disease is a chronic inflammatory bowel disease that effects as many as 1.6 million Americans. Current treatment involves the use of immunosuppressants to combat inflammation. These treatments can lead to unnecessary side effects and are extremely expensive. Emerging strategies to deliver immunosuppressive drugs locally involve a micro-droplet double emulsion that can be vaporized with ultrasound to release a water-soluble payload. Targeting ligands are attached to the droplet shell and selectively bind to the receptors MAdCAM-1 and P-Selectin that are over expressed in inflamed bowel endothelial cells. Studies were preformed in vitro and in vivo to assess the binding selectivity of P-Selectin targeted droplets.

OBJECTIVES

- Investigate the binding selectivity and affinity of sono-sensitive double micro droplet emulsions to the P-Selectin receptor.
- Examine the stability of the micro-droplet emulsion in-vitro
- Deterimine the binding selectivity of the P-Selectin targeted droplets in-vivo using an acute colitis model in rats.

METHODS

Laminar Flow Chamber



- A laminar flow chamber was used to pass both fluorescently tagged IgG control and P-Selectin targeted droplets over mouse BENd-3 cells stained with the Hoechst stain.
- Mouse BENd-3 cells were stimulated with TNF (tumor necrosis factor) to make the cells over express the P-Selectin receptor.
- Images were taken of each cell plate and the number of droplets per cell was determined
- A total of 18 trials were run. For each trial a total of 8 microscopy images were taken and the number of droplets to cells was determined.
- Two tailed T-Tests were used to determine whether the binding selectivity of each of the control methods was statistically significant when compared to the others.

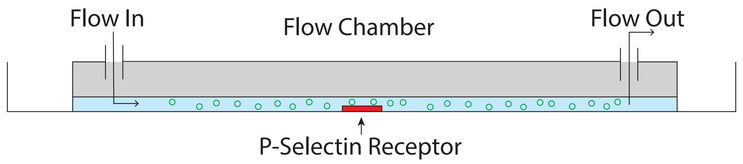
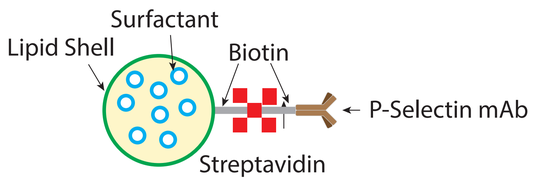


Figure 1. Schematic of the laminar flow chamber used to pass droplets over the P-Selectin receptor (Top). Schematic of the targeted micro-droplet double emulsion (Bottom).



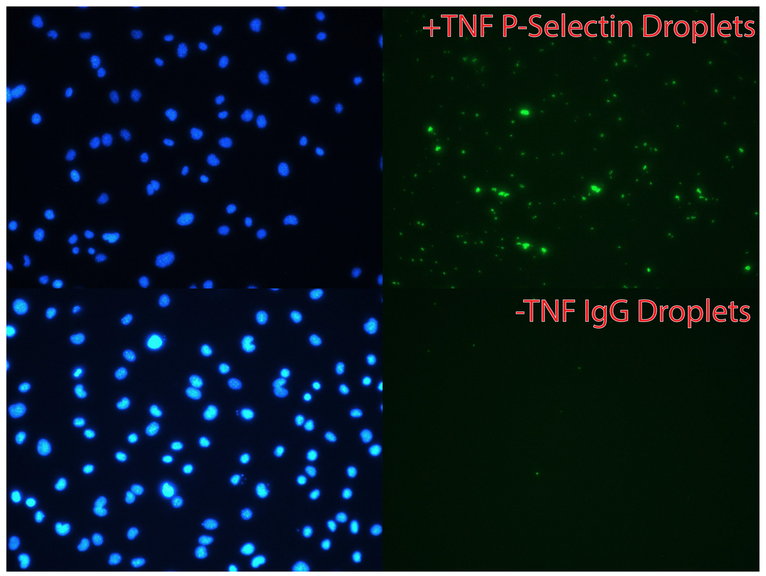


Figure 2. Two images of the same field of view for the two different control methods indicated above. Cell nuclei stained with the Hoechst Stain (left) and bound droplets (right).

Results

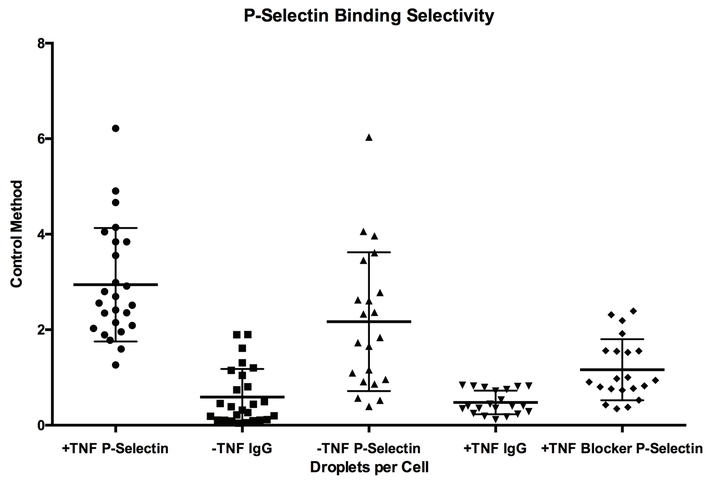


Figure 3. Droplets bound per cell for different control methods.

The droplets per cell counts of the +TNF P-Selectin trials were compared to control tests to determine the overall binding selectivity of the emulsion to the P-Selectin receptor.

The ratio of droplets to cells was approximately five times greater with P-Selectin targeted droplets than with the control droplets. The binding selectivity of the P-Selectin droplets was statistically significant when compared to control tests. As seen from the graph, the mean droplets/cell is much higher for the +TNF P-Selectin trials than any other trial.

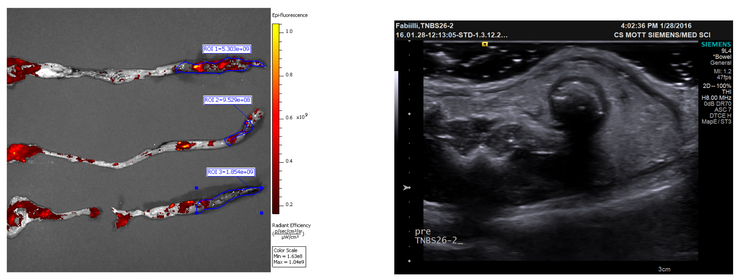


Figure 4. IVIS images showing the fluorescent intensity in the distal colon(left). An ultrasound of the colon showing the disease (right).

In-vivo experiments were performed to assess the binding selectivity of the microdroplets using an acute colitis model in rats. However the results are still inconclusive and require more trials.

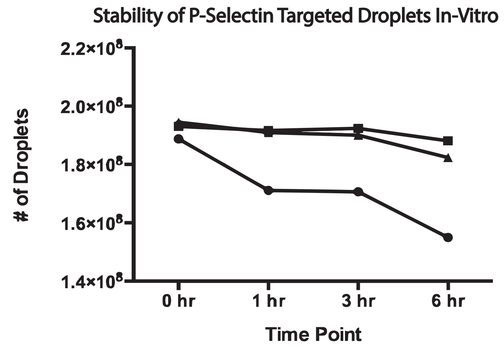


Figure 5. The size stability of the droplets was studied in-vitro. The number and mean diameter was determined at 0, 1, 3, and 6 hour time-points. The results were not statistically significant leading us to believe

Conclusions

The data collected let us conclude that P-Selectin targeted micro-droplet emulsions selectively bind in a statistically significant manner to cells stimulated with TNF. These in-vitro results lead us to believe that P-Selectin micro-droplet emulsions are a viable carrier for drugs which require spatiotemporal control during delivery.

Promising in-vitro results have prompted in-vivo experiments to examine the binding affinity and selectivity of the P-Selectin droplets using an acute colitis model in rats. The binding selectivity will be examined to see if the droplets are localized to the inflamed regions of the colon and not other organs after flowing through the bloodstream. These future experiments will use IVIS imaging to detect fluorescently labeled droplets in the infected colon, lung, liver, secum, and small intestine.

Acknowledgements

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