# RB n=54 05.03.19

Aim & Hypothesis

**Aim**

The aim of this study is to perform a screening of the immunomodulatory properties of Las, CHI3L1 agonist, using in vitro models of chemotaxis.

**Hypothesis**

In this first preliminary phase we evaluated if it is possible to study the chemotaxis of Neutrophils on the Zigmond chamber [Neuro Probe, Inc. Z02 Protocol - Visual Assay, Courtesy of Sally Zigmond].

Protocols

**Chambers:**

* Clean the chamber before each use. Wash it in warm water with tissue culture detergent, rinse well with distilled water and wipe dry. The bridge may also be wiped with 70% ETOH.

**Cells:**

* Place a drop of blood (from a finger prick) across the center of a 22mm x 40mm coverslip. Enough blood must be placed on each cover glass to allow it to clot and partially retract without drying. It takes close to 100μl per coverslip.
* Place the cover glass with blood in a moist chamber (a petri dish containing a wet piece of filter paper) at 37°C, with or without CO2.
* After about 45 minutes the blood clots and begins to retract, and fluid is visible around the edges. At this point the clot and red blood cells can be gently rinsed off with PBS. A monolayer of cells, mostly neutrophils, remains on the cover glass. Care must be taken not to let the cells dry*.*

**Incubation Medium:**

* Prepare a 10% gelatin stock by dissolving gelatin in boiling de-ionized water. Warm the stock to melt, and dilute it 1:10 with Hanks medium in which the bicarbonate has been removed and replaced with HEPES buffer (2.40 g HEPES/liter) at pH 7.2. Make sure the gelatin is really in solution when diluting with the Hanks. This medium is slightly acidic and slightly hypotonic. These conditions contribute to good cell locomotion; alkaline pH and hypertonicity are inhibitory.

**Chambers:**

* Rinse the cell layer on the cover glass with a few drops of this incubation medium. Quickly drain off the fluid on the coverslip, dry the ends of the cover glass with a Kimwipe, and invert the cover glass onto the chamber so that the cells lie over the bridge.
* Place the clamps on each side **without moving the coverslip**; any movement will lyse the cells on the bridge. The chamber assembly requires a little practice. With a minimal amount of fluid over the cells (but not allowing the cells to dry) the distance between the cover glass and the bridge will be very thin; 5 microns is optimal (the cells will appear slightly squeezed). If this gap is too large, the cells will not orient well. The gap can be measured by the difference in focal plane between the top of the bridge and the bottom of the cover glass, using the micrometer on the fine-focus knob of the microscope. With practice, you can lower the cover glass from one side. This helps eliminate air bubbles on the bridge; they can interfere with cell orientation.
* After the cover glass is secured, pipette 100μl of media into an open end of one of the grooves. Pipette 100μlof chemotactic factor (or cell-suspension media for a negative control chamber) into the other groove.
* Incubate at 37°C for 1 hour to allow the cells to respond.

**Cell Orientation:**

* Cell orientation is evaluated by observing the cells on the bridge (Using a 40X-phase objective). The orientation can be scored by the cell morphology. The front of a locomoting cell has a broad lamillipodum, while the tail is thinner and can be knob-like or drawn out into retraction fibers.

The orientation is easiest to score when the cells are moving well. If the chamber cools at room temperature for a while, the cells round up and are more difficult to score. The orientation should be scored at a specific location on the bridge, i.e., the chemoattractant side, the middle, or the media side. The level of orientation can vary greatly across the width of the bridge, depending on the concentration of chemoattractant. Scan a given chamber along the bridge until at least 100 cells have been scored.

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|  | **1st channel** | **2nd channel** |
| FBS | 100 μl of Medium | 100 μl of FBS |
| FMLP | 100 μl of Medium | 100 μl of medium with FMLP 100nM |
| LAS 10 μM | 100 μl of Medium | 100 μl of medium with LAS 1μM |
| FMLP and Las 10μM | 100 μl of medium with FMLP 100nM | 100 μl of medium with LAS 1μM |

**Results**

The volunteer is a healthy man