**Introduction**

- Macrophages play a central role in innate immunity as a first-line innate immune defense by migrating to inflamed tissues, where they clear microbes and repair injured tissues.
  - Classically activated cells (M1) play an important role in fighting intracellular pathogens and tumors.
  - Alternatively activated macrophages (M2) are involved in tissue repair, allergic reactions and tumor progression.
- THP-1 is an immortalized cell line used as a model to study monocytes and macrophages.
  - After exposure to PMA (phorbol-12-myristate-13-acetate), THP-1 cells start to differentiate into a macrophage phenotype with a flat and amoeboid shape.
- CSa is a protein fragment released from cleavage of complement component C5.
  - CSa is an anaphylatoxin, causing increased expression of adhesion molecules on endothelium and increased vascular permeability.
  - CSa is also a chemoattractant that plays a key role in increasing migration and adherence of neutrophils and monocytes to vessel walls.
- Traditional in vitro methods for studying Macrophage cell migration include:
  - **Microfluidic Chemotaxis Assay**: Researchers can see the cells, but they suffer from small gradients across the cell, low participation rates, and low throughput.
  - **Traditional Boyden Chamber Assays**: This predominant industrial approach has good throughput (96-wells). However, the researcher cannot easily visualize the process of cell migration, it requires many cells, and additional labeling or manual cell counting.

We have developed a new technology that eliminates most of the shortages of existing chemotaxis methods and provides a direct visualization of the migration process as it occurs.

**Obstacle**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Solution</th>
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<td>Low macrophage numbers in tissues and lack of proliferation in culture.</td>
<td>Requires 20 times less cells than traditional Boyden chamber protocols.</td>
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<td>Elaborated isolation and activation procedure.</td>
<td>Multiple experiments can be run simultaneously due to automated image acquisition and analysis.</td>
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<td>Low participation rate.</td>
<td>Negligible background noise with bottom-side metric.</td>
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**Incucyte ClearView Cell Migration Plate**

- **Visualize Chemotaxis**: The ClearView Plate incorporates an optically smooth membrane surface enabling acquisition of high-definition, phase-contrast images. Standard Boyden Chamber surfaces are not easily amenable to imaging.
- **Persistent Gradient**: The low porosity of the ClearView Plate results in a gradient that is stable for over 72 hours compared to 4 hours in traditional consumables.
- **Low Cell Density**: The combination of a long-term, persistent gradient and the interest in visualizing chemotaxis has resulted in an assay that requires significantly fewer cells compared to traditional Boyden Chamber Assays.
- **Integrin Signaling**: In the ClearView Plate, cells are required to migrate to the pores. This requires integrin interactions with the substrate that likely are not required in traditional Boyden Chamber consumables.
- **Label-free**: Measures cell migration without fixing, staining or cell scraping steps.
- **Automated Image Processing**: The unique design of the ClearView Plate facilitates quantitation of cells on top and the bottom of the membrane.

**Kinetic Measurements in Macrophage Chemotaxis**

- **100 nM CSa**: Macrophages were seeded at 2,000 cells per well on Matrigel-coated surface. A phase analysis (bottom-side) was performed, showing a concentration-dependent delay of macrophage response to CSa. Analysis of pharmacological response was performed at 4.5 hrs and 11 hrs. EC50 was calculated. Note that due to the initial delay, EC50 value changes significantly if the earlier time point is chosen. N=4. (A) Images acquired from the bottom side of the membrane were analyzed for the presence of cells. An image segmentation mask (blue for the cells, red for the pores) is blended with the original phase contrast image. After 4.5 hours of incubation a significant amount of cells can be seen on the bottom of wells with 111 nM of CSa (D), but not with 1000 nM of CSa (B). After 4.5 hours of incubation a significant amount of cells can be seen on the bottom of wells with 111 nM of CSa (B) but very little with 1000 nM of CSa (M). After 11 hours of incubation a significant amount of cells can be seen on the bottom of wells in both concentrations, with bigger cell number present in higher concentration (F and I). The observed delay with higher concentration of CSa was specific to Macrophage cells. When same concentrations were used in with Neutrophil cells, the delay was not detected (data not shown).

**Summary and Impact**

- While differentiated macrophage-like THP-1 cells had a strong concentration-dependent response towards CSa, no response was observed in THP-1 cells before differentiation, suggesting biological significance of monocyte maturation in CSa-mediated chemotaxis.
- Both M1 and M2-like cells were found to have a response towards a number of chemoattractants, with M1-like cells generally having a slower response as compared to M2-like cells.
- Macrophages displayed a lag phase before the cells start to migrate across the filter, which was longer with higher concentrations of chemoattractant.
- This lag is a function of the cells and not an artifact of the system, since other cell types do not display this behavior.
- The present study proves traditional single end time point assay to be insufficient for investigating Macrophage chemotaxis and provides new information about kinetics of macrophage migration.