Neutrophil Adhesion: A HTS Compatible Assay Using the Acumen eX3

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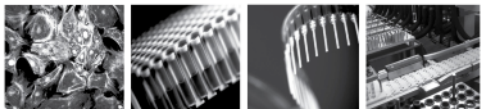
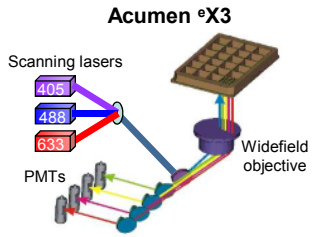


Introduction

A variety of pathological conditions exist in which cellular adhesion events are key to the evolving disease state. These include myocardial infarction, Adult Respiratory Distress Syndrome (ARDS), and general trauma. Cell adhesion also plays a crucial role in the immune system and is an important factor in inflammatory diseases such as rheumatoid arthritis, asthma and psoriasis. The concepts involved in the cell adhesion process are a rapidly developing area of cell biology and over the last five to ten years it has become possible to work out, at a molecular level, how cells attach to each other and to extracellular matrix molecules. This will be important for future drug development. The importance of cell adhesion molecules in the homing of lymphocytes to lymphoid organs, in neutrophil localization in inflammation, and in the interaction of both lymphocytes and neutrophils with vascular endothelium suggests that defects in these molecules might have severe consequences.

The Acumen eX3 is the fastest imaging system available, collecting and simultaneously analysing over 40 images/second, covering the entire well, without the trade off of having to use lower resolution. It offers up to three lasers from a range between 405nm to 633nm up to 4 channels of data collection per laser. The Acumen eX3 can be used to study the process of cellular adhesion, whereby adherent cells types specifically, endothelial cells can be grown to confluence in microtitre plate wells and other cells types e.g. neutrophils added. The neutrophils can be differentially stained with calcein AM and the adhesion profile monitored and quantified. Cell adhesion can be determined simply by correlating retained fluorescence with cell number. It is envisaged that this type of assay will be of use in the pharmaceutical screening of novel drugs directed against specific cellular adhesion molecules and in academia for the monitoring of the adhesion process in different disease states, such as myocardial infarction, Adult Respiratory Distress Syndrome (ARDS) and stroke *in vitro*. A simple, rapid, quantitative adhesion assay, routinely returning a signal to noise value of 6 to 1 has now been developed for the Acumen eX3 laser

1. Laser-Scanning Widefield Optics



The Acumen eX3 can sequentially scan with up to 3 lasers providing similar wavelength excitation to that of white light sources. PMTs detect up to 4 colours simultaneously. The application of laser scanning over a large area means that analysis is performed on an area, not a well basis. This equates to the simultaneous scanning of 4, 16 and 64 wells in 96, 384 and 1536 well format, respectively. Thus reconfiguration of assays into higher density plate formats results in a concomitant increase in throughput up to 300,000 samples per day in 1536 well microplates.

2. Protocol

A. Cell Culture Procedure

- Add 2,500 bovine arterial endothelial cells (BAEC) into each well of a 96 well plate and allow to grow to confluency over a period of 3 days.

B. Isolation of monocytes and neutrophils from "buffy coat" using Histopaque gradients

- Isolate neutrophils using standard isolation procedures and separation techniques (Histopaque gradients) as described in the literature.

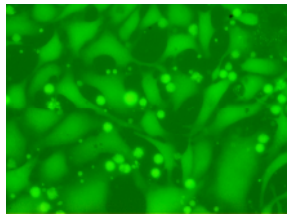
C. Dyes

- Calcein AM Supplied as 1 mg / mL (1 mM) solution in DMSO, aliquot and store at - 20° C (desiccation recommended)

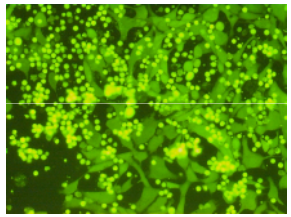
D. Adhesion assay procedure

- Neutrophils incubated with Calcein AM (5 µM final concentration) for 2 hours in serum free medium and washed 3x in serum free medium using centrifugation at 400xg. Cells re-suspended to give a cell count of 40,000 cells / mL.
- BAEC monolayers incubated with phorbol 12-myristate 13-acetate (PMA) (50 nM final concentration) contained in 100 µL serum free medium for 4 hours.
- BAEC monolayers washed 2x with warm serum free medium (37° C) with gentle aspiration.
- 4,000 neutrophils added per microtitre well in 100 µL of serum free medium.
- Neutrophils and BAEC monolayers incubated for 2 hours at 37° C.
- Microtitre wells washed 1x gently by aspiration with warm (37°C) PBS buffer.
- 100 µL of warm (37° C) PBS buffer added and the plate covered with a plate sealer and scanned on Acumen eX3.

3. Light microscope images of neutrophil adhesion to BAEC cells

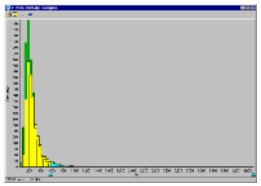


Adhesion of human neutrophils to control BAEC cells

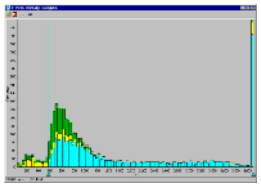


Adhesion of human neutrophils to PMA-stimulated BAEC cells

4. Simple Classification of Neutrophil Adhesion using Peak Intensity

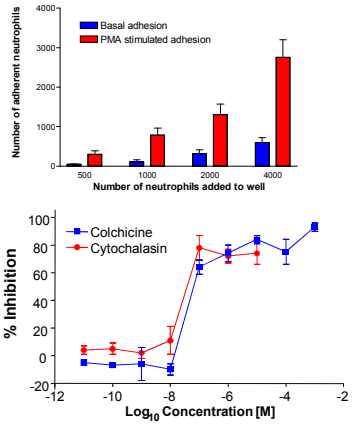


Basal adhesion



PMA-stimulated adhesion

5. Neutrophil adhesion to PMA-stimulated BAEC cells



Conclusion

- High content and high throughput cell based assays are not mutually exclusive
- Acumen eX3 is a compatible instrument for rapid high content neutrophil adhesion drug screening assays.
- Similar application of Acumen object characteristic algorithms could be potentially applied to other types of adhesion assay.

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