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Simplifying Progress

High Throughput Flow Cytometry: Monitoring Osmolarity using Cell Mimic Polymer Beads

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Introduction

In high throughput flow cytometry screening, hours can pass between the analysis of the first plates in queue relative to those at the end. Monitoring culture conditions e.g., O_2, pH, CO_2, [glucose], [amino acids], *etc.* is crucial for well-controlled comparisons among experimental groups throughout the analysis. Although it can profoundly affect cell health, changes to osmolarity (salinity) are not typically recorded, nor is there a way to measure this in a high throughput format. Here we demonstrate an in-well, high throughput-compatible, bead-based (cell mimics) technique to measure the relative osmolarity of culture media contemporaneously with all other measures. The cell mimics can also be added into "sentinel" wells at the leading and tailing ends of the plates being analyzed monitoring evaporation losses across a single assay. The cell mimics are made with tunable optical properties such that in low salt the polymer relaxes and the scatter of the encapsulated material decreases. In higher salt, water is removed from the polymer, pulling the encapsulated scattering material closer leading to greater internal reflections/scatter.

1. Methods

- Osmolarity sensitive cell mimics were generated in collaboration with Slingshot Biosciences using their proprietary, polymer-based cell mimic technology.
- A flow cytometry assay was established using these cell mimics. Standard curves were generated in both water and culture medium with the addition of increasing amounts of concentrated phosphate buffered saline (PBS).
- Mouse hybridomas were exposed to hyperosmotic conditions for 24-hours and apoptosis was measured by caspase 3/7 activation. Osmolarity cell mimics were added to the cells to be analyzed after incubation with the caspase substrate, immediately prior to analysis on the flow cytometer.

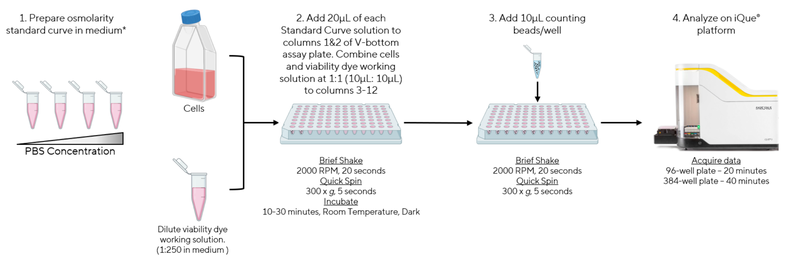

Figure 1: **Schematic representation of an osmolarity bead protocol.** The osmolarity sensitive beads can be used with many assays. Shown here is a protocol where they are used with the iQue® Cell Count and Viability Kit. Portion of figure made with BioRender.

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2. Gating and Detection Strategy

Here, flow cytometry analysis is performed on a Sartorius iQue®3 instrument. However, any flow cytometer equipped with scatter detection may be used. Standard gating is used to identify and then focus on mono-dispersed beads. Scatter was measured from whole gated populations to avoid any biases.

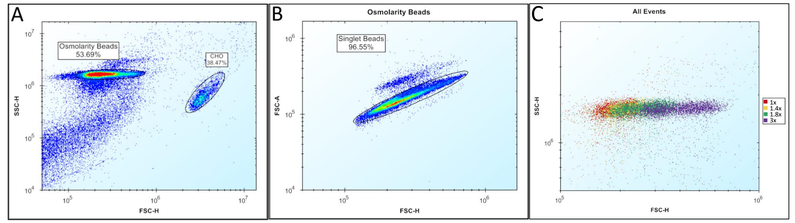

Figure 2: **Gating hierarchy and scatter detection of cell mimic beads in varying concentrations of PBS.** The gating hierarchy in ForeCyt for osmolarity mimics includes A) identifying the cell mimic population using FSC-H and SSC-H. B) Single cell gate applied to exclude any doublets. With differential scattering C) notable shifts in forward scatter is noted with varying salt concentrations in a dose-dependent, linear manner.

3. Sensitive - Detect Evaporation in as few as 15 minutes

The osmolarity sensitive cell mimics were also able to detect changes in osmolarity due to evaporation in the wells of an open 96-well plate at room temperature. It is noteworthy that after only 15 minutes, a measurable, statistically significant ($p<0.05$) increase in osmolarity was detected.

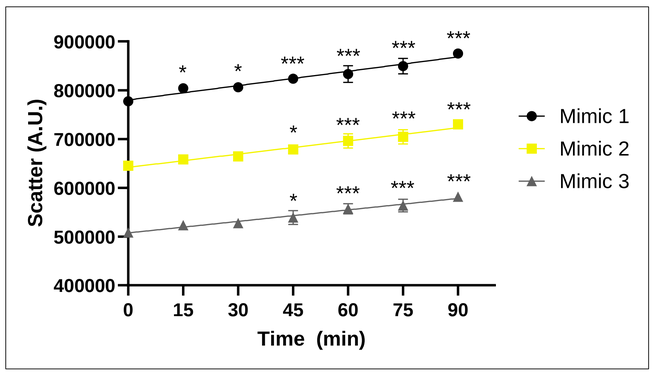

Figure 3: **Osmolarity sensitive cell mimics demonstrate a linear response to the increasing osmolarity as liquid is lost due to evaporation in a 96-well plate.** An uncovered 96-well plate containing osmolarity beads in 1x PBS was placed on the iQue®3's plate holder. Every 15 minutes, the same plate was shaken and a new set of three wells was analyzed. (* p <0.05, ** p<0.01, *** p<0.001)

4. Consistent - Linear Response Across Cytometers

Whether using the iQue®3 high throughput flow-cytometer or a traditional flow cytometer, qualification of osmolarity for a dilution series resulted in a linear response on across multiple assessments. The R^2 value for the mean results was greater than 0.99 on both instruments (Figure 4), demonstrating the robustness of the assays across multiple flow cytometers.

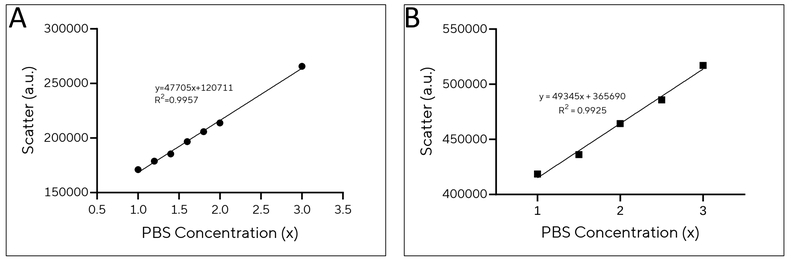

Figure 4: Results of PBS linear dilutions on multiple instruments. The linearity and slope of the response to increasing PBS concentration in water using the (A) iQue® high throughput cytometer is nearly identical to the response of (B) PBS in culture media in a traditional flow cytometer.

5. Predictive - Early Warning of Hyperosmolarity-Induced Apoptosis

Hybridoma cells were grown in a hyperosmotic state for 24-hours by adding a titration of 10x PBS. Apoptosis was observed by and increase in Caspase 3/7 activity. Hybridomas appear to be stable in a slightly hyperosmotic environment but fall at a notable threshold resulting in rapid increases in apoptosis and cell death.

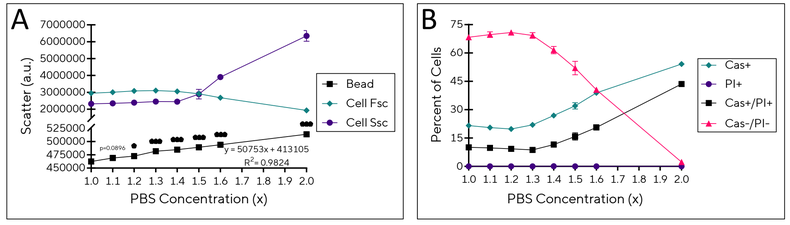

Figure 5: **Osmolarity beads were used in an analysis of hybridoma cells grown under hyperosmotic conditions.** A) Scatter for the cells and the beads was analyzed in hyperosmotic cell growth conditions. (B) Cell health of the hybridomas was monitored with a labeled Caspase 3/7 responsive dye and the membrane integrity dye propidium iodide (PI).

6. Conclusions

In cell culture there are many factors affecting cell health that can confound experimental results. Here we have demonstrated an in-well cell mimic that can detect small changes in osmolarity within typical flow cytometry experiments. The response and sensitivity of the cell mimics allows for rapid analysis of osmolarity to flag plate-to-plate and well-to-well variability in high throughput flow cytometry.