NANObiose
Smart Tools for Cell Biology

Static and Dynamic Cell Viability Analysis in SecretCells® by Resazurin Endpoint Assay

Objectives

SecretCells® is a microfluidic lab-on-a-chip system designed for continuous perfused 2D or 3D cell culture, efficiency and/or safety evaluation of molecular entities of interest. As shown previously, continuous perfusion culture in SecretCells® microfluidic device does not affect the viability of cells (*Nanobiose, Application Note 001/2021*).

This application note describes two methods (static and dynamic) of monitoring and analysing cell viability on SecretCells® microfluidic device with Resazurin endpoint assay by evaluating the viability of human macrophage cells exposed to lipopolysaccharide (LPS) treatment.

Materials and Experiment Setup

- **Cell Model:** Macrophages generated from the human monocytic cell line THP-1 using 10 ng/mL Phorbol 12-myristate 13-acetate (PMA, Sigma P8139) for 48 hours.

- **Culture Scaffolds:**
  - *Nitrocellulose membranes* (hydrophilic, 0.22 µm pore size; Millipore, GSWP04700). Cells were directly seeded onto the membrane at 2×10^4 cells/cm^2 (30 000 cells/well).
  - *CultiSpher®-G macroporous gelatin-coated microcarrier beads* (M9418 - Sigma-Aldrich). 2.5 mg beads per well with 50 000 cells/well.
  - *3D Biotek 3D Insert™ Polystyrene (PS) scaffold for 96 well plates*: used according to the manufacturer's instructions; i.e., one insert with 125 000 cells per well.

- **SecretCells® System:** assembled and used according to Nanobiose's instruction manual. The cells were seeded under static conditions and subsequently constantly perfused at 5 µL/min with 100 ng/mL of LPS in culture medium for 6 hours followed by additional 12 hours with culture medium, all within a 37°C and 5% CO2 environment.

As a parallel control, cells were seeded in 96-well culture plates under the same conditions and stimulated with 100 ng/mL LPS for 18 hours under standard static culture conditions.

- **Cell viability analysis:** was monitored and evaluated at the end of the experiment using Resazurin assay as follows:

  - *Static Viability analysis:* was performed by disassembling the SecretCells® system and adding the rezasurin dye directly to the wells - as with a standard culture in plates.
  - *Dynamic Cell Viability analysis:* was performed under perfusion by loading the resazurin dye solution at 5 µL/min and collecting the cell supernatants in effluents from wells. They were subsequently transferred to new wells of a 96-well cell culture plate to record the fluorescence intensity of converted resorufin.

For more details, please see "*Cell viability Analysis by Resazurin Endpoint Assay*" protocol from Nanobiose.

Results

The effect of LPS on human THP-1 derived macrophage cell viability in SecretCells® lab-on-a-chip system was determined as the percentage difference in reduction of resazurin to resorufin between LPS-treated cells and reference cells (no LPS added). Exposure of THP-1 macrophage cells to LPS under perfusion resulted in differing effects on cell viability depending on the scaffold used, as measured by static viability analysis method.

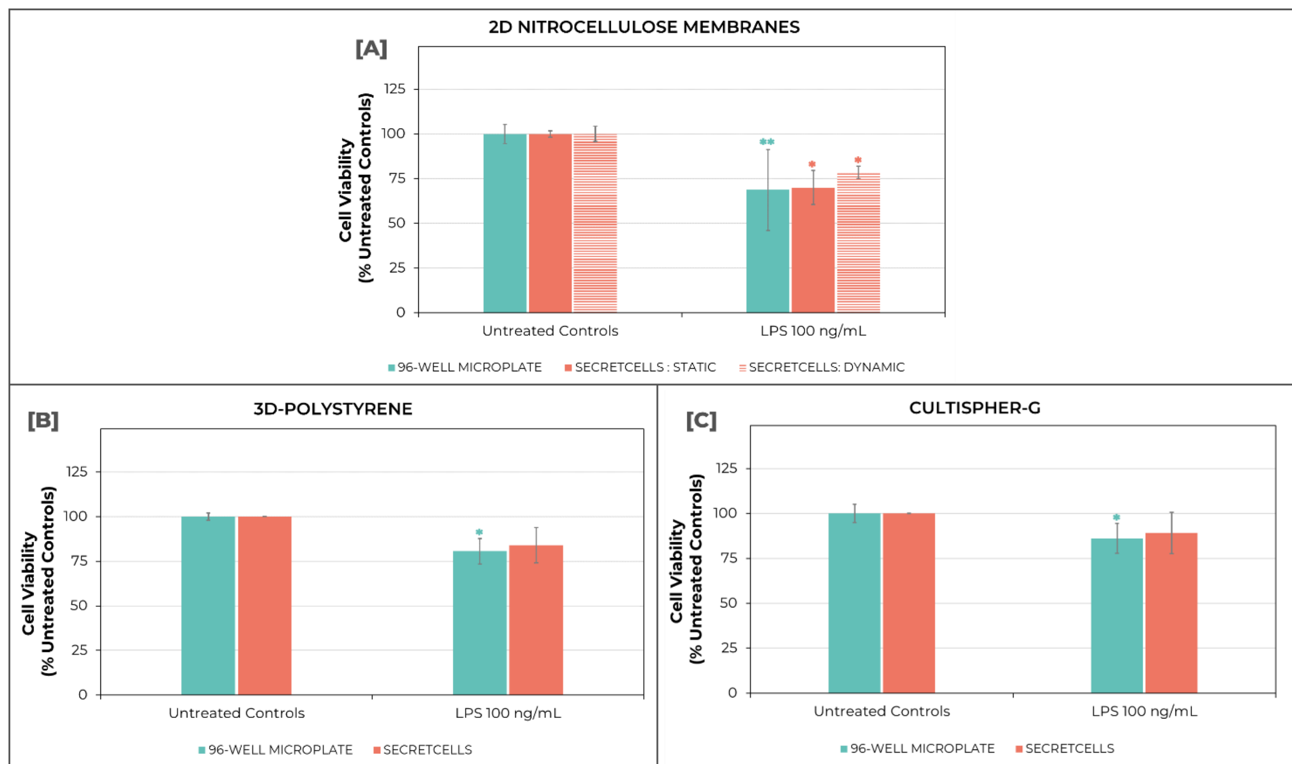

Figure 1: THP-1 derived macrophage cell viability after 18 hours under perfusion with 6-hour LPS treatment in SecretCells® microfluidic system compared to static culture in plate (18-hour LPS treatment). * $p<0.05$; ** $p<0.001$ (ANOVA & t-test)

2D monolayer cells on nitrocellulose membranes showed around 30% decrease in cell viability ($p<0.05$; Figure 1-A) that was comparable to parallel cultures under static conditions but which, in contrast, showed a greater variability ($p<0.001$; Figure 1-A). In 3D configurations, there was less than 20% decrease in cell viability for cells within porous polystyrene 3D scaffold ($p<0.05$; Figure 1-B) and about 10% in CultiSpher®-G gelatin microcarriers ($p>0.05$; Figure 1-C). Their level of cell viability was comparable to parallel equivalent cultures under static conditions with, nevertheless, a significant decrease vs. untreated controls ($P<0.05$; Figure 1-B/C) and a slightly better viability in SecretCells® but the difference was not statistically significant ($p>0.05$).

Furthermore, the two approaches of measuring cell viability in SecretCells® showed an overall good concordance for 2D monolayer cells on nitrocellulose membranes (Figure 1-A). In fact, cell viability measurement by dynamic analysis method resulted in slightly

less than 25% decrease in LPS-treated cell viability vs. untreated controls ($p<0.05$; Figure 1-A). The difference between the two methods was not statistically significant ($p>0.05$).

Conclusion

This study indicates the efficacy and reliability of the two methods of measuring the cell viability in SecretCells® with Resazurin Endpoint Assay. The results demonstrate that it is possible to monitor and evaluate the impact of molecular entities of interest on the overall viability of cells under perfusion in SecretCells®.

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