**Callisto™ In Vitro Cell Culture System: Application and Reproducibility to Study Small Cell Populations.**

Awo D. Osafo-Addo¹, Maria Haslip¹, Anup Srivastava¹, Ying Sun¹, Patty J. Lee¹, Tingxia Guo², Michael Watson², Arielle Mulenos¹, Naga Gopi Devaraju², Haibiao Gong², Nianzheng Li² and Jonathan Koff³

1. Section of Pulmonary, Critical Care, and Sleep Medicine, Yale University School of Medicine, New Haven, CT, USA
2. Fluidigm Corporation, South San Francisco, CA, USA

**BACKGROUND**

Callisto™ (Fluidigm, South San Francisco, CA) is an automated microfluidic platform for manipulation of multiple cell conditions in small numbers (e.g., 300 - 1500 cells per chamber). We have developed protocols to culture, stimulate, stain, harvest and lyse cells within the chambers of the integrated fluidic circuit (IFC) at user-defined time-points. Here, we investigate the ability of Callisto™ to provide enough cellular material for analysis after cell culture experiments (e.g., RNA and protein) and also to show that the system is amenable to long term in vitro cell culture and viral transduction experiments.

**METHOD**

Callisto™: Callisto™ is an automated system with environmental controls that uses a specialized integrated fluidic circuit (IFC) and experimental design software.

**Cell Culture Conditions:** Human airway epithelial cells line (BEAS-2B), human monocytic cell line (THP-1) and primary mouse lung endothelial cells (MLEC) were used to test experimental outcomes. Callisto™ was programmed to dose and collect cellular material at different time points for:
1) Cell stimulation with lipopolysaccharide (LPS) and Interferon-gamma (IFN-γ).
2) Lentiviral transduction of gene overexpression and silencing.
3) Adenoviral vector delivery to determine nuclear localization. Data analysis includes qRT-PCR (lysates) and V-PLEX™ (Mesoscale Discovery) for cell supernatants.

**CONCLUSIONS**

1) Cell culture protocols have been established for BEAS-2B, THP-1 cell line and MLEC (primary cells) in Callisto™
2) Experiments provide cell culture lysates and supernatants from ~300-1500 cells for the following applications:
   - Gene expression analysis
   - Viral transduction experiments
   - Cytokine quantification.

Therefore, Callisto™ is a reliable, automated cell culture system that allows for mRNA and protein analysis in small cell populations.

**FUTURE DIRECTIONS**

- Continue to standardize cell culture conditions for different cell types (e.g., PBMC and primary epithelial cells).
- Couple Callisto™ to other applications, such as CyTOF®

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**Cell Culture Conditions**

**Fibroblasts**

**Airway epithelium**

**Endothelium**

**Monocytes**

**1h Post-Attachment**

**24h in culture**

**Gene expression analysis**

**FGF2 - Beas2b**

**IL12p70 - Beas2b**

**TNFα - Beas2b**

**IFNγ - Beas2b**

**IL13 - THP1**

**IFNA16 - THP1**

**IFNB1 - THP1**

**INS - THP1**

**IL9 - THP1**

**IL6 - Beas2b**