Simplifying Stem Cell Characterization and Analysis with a Personal Flow Cytometer

Mirko Corselli, PhD
Supervisor, R&D
Flow cytometry is essential for our understanding of the hematopoietic system. We have not fully utilized the power of flow cytometry to address biological questions in other systems.
Challenges:

- Multiple methodologies commonly used in stem cell research; time and material consuming

- Unable to correlate expression of surface and intracellular molecules

- Difficult to quantitate percentage of cells expressing markers of interest

**Methods of Analysis**

<table>
<thead>
<tr>
<th>Cell Surface Markers</th>
<th>Flow Cytometry</th>
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<tr>
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<tr>
<td>Intracellular Proteins</td>
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<td>Fluorescent proteins</td>
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<td>Cellular Processes</td>
<td>Multiple</td>
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Flow Cytometry in Stem Cell Research

Discovery

Analysis

Isolation

CD200

HLA-A,B,C

NSC

Neurons

CD200

BD
Overview

• Stem Cell Surface Marker Analysis and Discovery

• Intracellular Flow Cytometry Applications
  • Cell preparation and procedural considerations

• Streamlining Setup and Analysis of Experiments
Stem Cell Surface Marker Analysis and Discovery
Pluripotency can be monitored by a variety of assays:

- Morphology
- In vitro and in vivo differentiation potential
- Cell surface and transcription marker expression
  - ICC
  - Flow Cytometry
Pluripotent human embryonic stem cells (hESCs) express SSEA-3 and TRA-1-81 and do not express SSEA-1.
Induced pluripotent stem cells (iPSCs) derived from fibroblasts express TRA-1-60 and SSEA-4 and no longer express CD13.
Enabling researchers to immunophenotype cell populations by flow cytometry or immunofluorescence microscopy using BD’s portfolio of monoclonal antibodies

<table>
<thead>
<tr>
<th>Product</th>
<th>Contents</th>
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<td>• 242 CD markers* • Isotype controls • Alexa Fluor®→ 647 second step</td>
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*CD and other cell-surface molecules. One marker per well.
Cell-Surface Marker Signatures for the Isolation of Neural Stem Cells, Glia and Neurons Derived from Human Pluripotent Stem Cells

Shauna H. Yuan, Jody Martin, Jeanne Elia, Jessica Flippin, Rosanto I. Paramban, Mike P. Hefferan, Jason G. Vidal, Yangling Mu, Rhiannon L. Killian, Mason A. Israel, Nil Emre, Silvia Marsala, Martin Marsala, Fred H. Gage, Lawrence S. B. Goldstein, Christian T. Carson

1 Howard Hughes Medical Institute and Department of Cellular and Molecular Medicine, School of Medicine, University of California San Diego, La Jolla, California, United States of America, 2 Department of Neurosciences, School of Medicine, University of California San Diego, La Jolla, California, United States of America, 3 BD Biosciences, La Jolla, California, United States of America, 4 Anesthesiology Research Laboratory, Department of Anesthesiology, University of California San Diego, La Jolla, California, United States of America, 5 Laboratory of Genetics, The Salk Institute for Biological Studies, La Jolla, California, United States of America, 6 Biomedical Sciences Graduate Program, University of California San Diego, La Jolla, California, United States of America, 7 Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovakia
Screening Workflow: Flow Cytometry

Prepare a single-cell suspension and aliquot into three 96-well plates

Reconstitute BD Lyoplates

Transfer reconstituted antibodies to plates with cells

Incubate Wash Secondary antibody Wash Fix Cells/Wash Collect data

Analyze data
Surface Marker Screening of MCF-7 Cells Using the BD Accuri™ C6

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**Gate: No gating**

**Gate: P1**
### Comparable Screening Results Using BD Accuri C6 or BD FACSCanto™ Flow Cytometers

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Efficient and Scalable Purification of Cardiomyocytes from Human Embryonic and Induced Pluripotent Stem Cells by VCAM1 Surface Expression

Hideki Uosaki¹,², Hiroyuki Fukushima¹,², Ayako Takeuchi³, Satoshi Matsuoka⁴, Norio Nakatsuji⁵, Shinya Yamanaka⁶, Jun K. Yamashita¹,²

SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells

Nicole C Dubois¹, April M Craft¹, Parveen Sharma², David A Elliott³, Edouard G Stanley³, Andrew G Elefanty³, Anthony Gramolini² & Gordon Keller¹

Differential Expression of Surface Markers in Mouse Bone Marrow Mesenchymal Stromal Cell Subpopulations with Distinct Lineage Commitment

Maria Rostovskaya, Konstantinos Anastassiadis*
Intracellular Flow Cytometry
Applications for Stem Cell Biology
• Stem cells are often defined by the expression of key master gene regulators.

• Intracellular flow cytometry allows for a quantitative measure of stem cell differentiation.
Endodermal Differentiation from hESCs
Western Blot and Flow Cytometry: Endodermal Differentiation from hESCs
Overview of Intracellular Staining

Allows for the detection of transcription factors, cytoplasmic, and/or phosphoproteins, and cytokines by flow cytometry.
Permeabilization Buffer Choice: Monitoring Neuronal Differentiation with Intracellular Flow Cytometry

Identify Sox1, Pax6, Sox2, and doublecortin expressing cell populations by intracellular flow cytometry

- Selection of appropriate permeabilization buffer system
Permeabilization Buffer Choice: Monitoring Neuronal Differentiation with Intracellular Flow Cytometry

**Sox1 PE Sox2 PerCP-Cy5.5 Pax6 Alexa Fluor® 488**

- Red = BD Phosflow™ Perm Buffer III
  - DCX PE: Shows expected staining
- Blue = BD Pharmingen™ Transcription Factor Buffer Set
  - Sox1 PE: Better resolution
  - Sox2 PerCP-Cy™ 5.5: Better resolution
  - DCX PE: Poor resolution
Confirm DCX expression by co-staining neuron differentiation cultures with Pax6 using BD Phosflow Perm Buffer III.
Combining Surface and Intracellular Staining

- Simultaneous staining of surface and intracellular markers
  1) Fixation
  2) Permeabilization
  3) Simultaneous cell surface and intracellular staining

- Shorter protocol
- Surface marker must be amenable to post-fixation staining
Combining Surface and Intracellular Staining

- Sequential staining of surface and intracellular markers
  1) Cell surface stain
  2) Fixation
  3) Permeabilization
  4) Intracellular stain

- Widely applicable to surface markers
- Assumes fluorochrome is stable in permeabilization buffers
Stem Cell Kits on the BD Accuri C6

Stem cell kits include:

- Conjugated antibodies
- Buffer systems
- Isotype controls
- Compensation particles

BD Accuri C6

- Easy to use
- 2 lasers – 6 parameters
- Fixed voltages
- Cell counting
- Continuous sampling
- Kits and templates
Pluripotent hESCs express Oct4 and SSEA-4 and do not express SSEA-1.
Pluripotent hESCs express Oct4, Nanog, and Sox2.
Define a cell surface signature for cardiac progenitors using BD Lyoplate™ Cell Surface Marker Screening Panel

- Intracellular flow on hESC-derived cardiac progenitors was performed to identify a correct time-point for screening.
Intracellular Flow Cytometry: Cardiac Differentiation

Day 0

Day 2

Day 3

Day 4

Day 5

Day 6

Day 7

Day 8

Karina Palomares, Chen and Evans Laboratories, UCSD
Monitoring Apoptosis, Cell Cycle, and DNA Damage in Stem Cell Cultures

- Monitoring cellular processes in different stem cell populations
  - hESCs
  - hESC-derived neurons

- BD Pharmingen™ Apoptosis DNA Damage and Cell Proliferation Kit
  - Proliferation (BrdU PerCP-Cy™5.5)
  - DNA Damage (pH2AX Alexa Fluor® 647)
  - Apoptosis (Cleaved PARP PE)
  - Fixation and Permeabilization Buffers
Flow Cytometry to Monitor Four Parameters:

- **Pluripotency Status**
  - Nanog Alexa Fluor® 488
- **Proliferation**
  - BrdU PerCP-Cy™5.5
- **DNA Damage**
  - pH2AX Alexa Fluor® 647
- **Apoptosis**
  - Cleaved PARP PE

BD Pharmingen™ Apoptosis DNA Damage and Cell Proliferation Kit
Nanog$^+$ cells have higher levels of pH2AX and are more proliferative than Nanog$^-$ cells.
Flow Cytometry to Monitor Four Parameters:

- **Differentiation Status (NSC vs Neurons)**
- **Sox2 Alexa Fluor® 488**
- **Proliferation**
  - **BrdU PerCP-Cy™5.5**
- **DNA Damage**
  - **pH2AX Alexa Fluor ® 647**
- **Apoptosis**
  - **Cleaved PARP PE**
Sox2− cells (Neurons) do not proliferate and have lower levels of pH2AX.
Streamlining Setup and Analysis of Experiments
Mesenchymal Stromal Cell Definition

- MSCs must be plastic adherent.
- MSCs must express CD73, CD105, and CD90 and lack expression of CD45, CD34, CD14, CD11b, CD79a, and CD19.
- MSCs must differentiate into osteoblasts, adipocytes, and chondrocytes in vitro.
Analysis of Human BM MSC
Stem Cell Kits and Templates on the BD Accuri C6

• Free downloadable BD Accuri C6 Software Templates are matched to each kit.
• Kit and templates lead to quick and easy setup and analysis of stem cell populations.

### Stem Cells

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<th>Category</th>
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<th>Brand</th>
<th>Kit</th>
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A BD Accuri C6 template is a pre-defined workspace that includes gates, labels, run criteria, and compensation settings for a specific assay.
BM MSC Analyzed Using Template

BD Accuri™ C6

Select plot type to make a new plot.
Conclusions

• Stem Cell Surface Marker Analysis and Discovery
  • Phenotypic characterization
  • Discovery of novel cell surface marker signatures

• Intracellular Flow Cytometry Applications
  • Cell preparation and procedural considerations
  • Monitoring and quantitating pluripotent and lineage specific differentiation and cellular processes

• Streamlining Setup and Analysis of Experiments
  • Use of BD reagents, kits, and templates on BD Accuri C6
Characterizing Stem Cell Populations

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- One rapid methodology for broad stem cell applications
- Ability to correlate expression of surface and intracellular molecules
- Ability to quantitate percentage of cells expressing markers of interest
Research Solutions for Stem Cell Biology

- Free downloadable templates
- Broad reagent portfolio
- Product information sheets
- Technical documents
- Webinars
- BD Accuri™ News

www.bdbiosciences.com/resources/accuri
Acknowledgements

BD Biosciences:

San Diego
  – Christian Carson
  – Nil Emre
  – Rosanto Paramban
  – Jason Vidal

San Jose
  – Ranga Partha
  – Andy Wang
Questions?