Fluorescent labeling of extracellular markers on stem cells from human breast milk

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Introduction

Breastfeeding is regarded as the most nutritious and health-sustaining form of feeding for developing individuals of various mammalian species, and breast milk has evolved to suit the specific needs of the infant of a given species. The consumption of maternal breast milk by the infant positively influences their development and immunity. Effects of breastfeeding on infants likely play a role in promoting comprehensive wellness that persists into adulthood and sustained over a lifetime. This has largely been attributed to the lymphocyte-based immunity that is passed from mother to offspring during breast feeding. It was previously believed that the largest cellular fractions present in human breast milk consisted of immune cells and epithelial cells. Recently, flow cytometric data revealed that non-immune cells in healthy mothers and infants are the dominant cell type. Among these cells are pluripotent multi-lineage stem cells.

Like maternal immune cells, breast milk stem cells survive the infant GI tract and enter systemic circulation. Their pluripotent characteristics suggest that they are influential in development and restoration of many cell types. Developing infants have specific nutritional and developmental needs and the breast milk they receive from their mother is tailored to satisfy their individual requirements. Furthermore, preterm infants have added needs as their susceptibility to disease and mortality rates are increased.

Pumping breast milk and freezing it is common practice in hospitals, neonatal intensive care units (NICU), and in numerous households. Freezing milk may alter the cellular constituency of breast milk by reducing the amount of viable stem cells. We are interested in the alteration of stem cell viability and concentration in human breast milk frozen at varying temperatures and durations. This reduction may negatively impact infant development; vulnerable populations such as preterm infants have higher nutritional requirements and less volumetric capacity in their stomach. Freezing milk may undermine attempts to supply preterm infants with the nutritional requirements needed to sustain life. What follows is the development of an assay to identify pluripotent stem cells in human breast milk.

Aims

- This work aims to develop optimal antibody dilutions for the extracellular stem cell markers CD34, CD45, TRA 1-81, and SSEA-5 on pluripotent stem cells from human breast milk.
- With this single stain data, staining index for each antibody in their respective channel will be calculated. Staining index is a measure of resolution sensitivity and is used to determine maximum fluorescence of a fluorophore on a specific instrument.

Experimental Design

- Fresh breast milk from a two month post-partum mother was analyzed
- Cells were prepared and plated at $1x10^6$ cells/mL
- Cells were stained with viability fluorophore eFlour450, CD34 fluorophore PE-Cy 7, CD45 fluorophore PerCP-eflour 710, TRA 1-81 fluorophore APC, and SSEA-5 fluorophore PE. An unstained population of cells was used for a negative control.
- Respective channels were used to read the aforementioned fluorophores: Violet 2 channel for viability, PE-Cy 7 channel for PE-Cy 7, Violet 1 channel for PerCP-eflour 710,
- APC channel for APC, and, PE channel for PE.
- Cells were analyzed in the Cell Sorting and Flow Cytometer Core at Colorado State University using CyAn3, a three laser Beckman Coulter CyAn ADP flow cytometer.
- Analysis was performed using FlowJo software.

Results

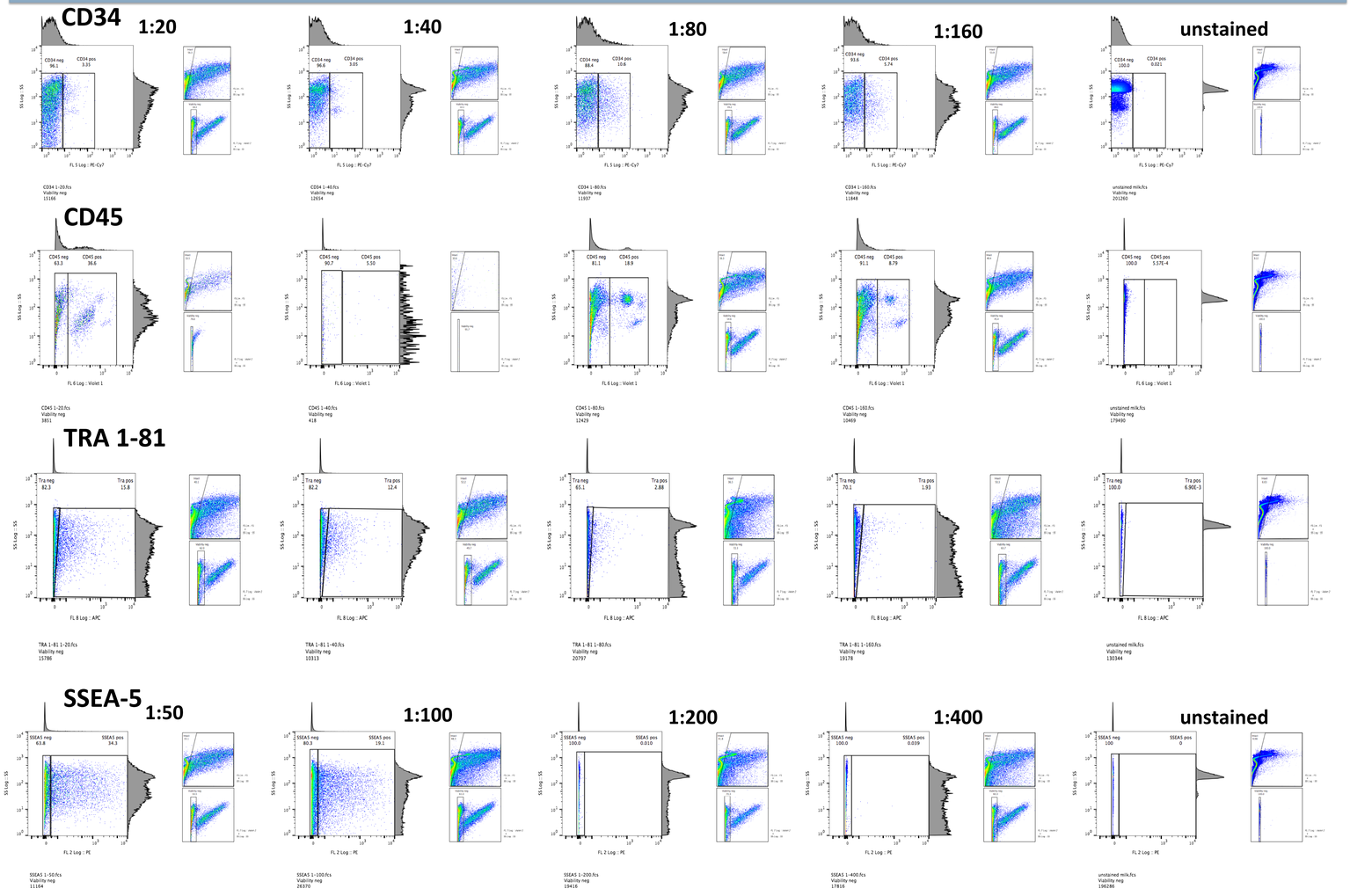

Figure 1: Single stain data for extracellular stem cell markers CD34, CD45, TRA 1-81, and SSEA-5 in a cell population from human breast milk plus an unstained negative control. FlowJo gating strategy: Intact cells ➔ Viability Negative ➔ Antibody neg & pos. Dilutions are 1:20, 1:40, 1:80, and 1:160 for CD34, CD45, and TRA 1-81. SSEA-5 followed a 1:50, 1:100, 1:200, and 1:400 dilution. Optimal dilutions are as follows: CD34 and CD45 – 1:80, TRA 1-81 -1:20, and SSEA-5 – 1:100.

Results cont.

	Staining Index Calculation	Flow Cytometry Data Results
CD34	1:80	1:80
CD45	1:80	1:80
TRA 1-81	1:20	1:20
SSEA-5	1:400	1:20

Table 1: A comparison of a calculated staining index and flow cytometric data. Staining index is defined as:

$$[MFI_1 - MFI_2]/2 \times SD_{neg}$$

where MFI_1 is the mean fluorescence intensity of the positive population, MFI_2 is the mean fluorescence intensity of the negative population, and SD_{neg} is the standard deviation of the negative population. Mathematical calculation confirmed three of four dilution concentrations produced by flow cytometry.

Conclusion and Future Direction

The next step in optimizing this detection assay will include another single stain along with a fluorescence minus one (FMO) stain where cells will be prepared and stained with four of the five antibodies in our panel in multiple combinations. This is done to eliminate fluorescent spillover of a fluorophore in multiple detection channels.

The main aim of this research revolves around common breast milk freezing practices and their effect on the cellular fraction of the milk, specifically the effect freezing has on stem cell viability and concentration. Preliminary data has shown reduced viability and concentration of stem cells from breast milk frozen at -20°C. Preterm infants are a vulnerable population that require additional care after birth as they are unable to hold as much milk as an infant that went to full term. If freezing milk drastically reduces viability and concentration of cells, the preterm infant would be further disadvantaged.

In moues models induced pluripotent stem (iPS) cells have shown the ability to differentiate into T-lymphocytes. This differentiation of iPS cells has vast implications for regenerative medicine and in the reconstitution of the immune system. To induce these cells a OP9-DL1 culture system is used to promote the differentiation of stem cells to antigen-specific T-cells. We are interested in sorting and culturing stem cells from human breast milk iand determining their T-lymphocyte differentiation capabilities.