Kinetic stem cell counting: A computational simulation technology for quantifying therapeutic tissue stem cells and their expansion kinetics during biomanufacturing

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ARMI ROADMAP FOCUS

The main goal of the project was to develop new software that would **automate** the derivation and validation of mathematical algorithms for rapid counting of tissue stem cells in cell and tissue biomanufacturing processes and products. The tissue stem cell-specific fraction (**SCF**) is predicted to be a **critical quality attribute (CQA)** for the four modular stages of the **Cell Culture & Harvest** segment of the ARMI|BiofabUSA Roadmap:

Tissue/Cell Harvest
Cell Banking
Expansion Culture
Cell Harvest and Wash

PROJECT GOALS

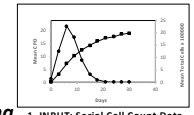
In 2020, Asymmetrex® reported the first method for specific quantification of therapeutic tissue stem cells in complex tissue cell preparations.[1-4] The method, called **kinetic stem cell (KSC) counting,** uses computational simulation to quantify the specific fractions of stem cells, committed progenitor cells, and arrested differentiated cells in tissue cell cultures. The input for the analysis is conventional cell count data. One of the outputs, the stem cell-specific fraction with serial culture, can be used to derive mathematical algorithms that convert 72-hour cell population doubling time data directly into the stem cell-specific fraction.

The main goals of the project were:

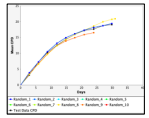
1. Automate the rapid-counting algorithm derivation process.

2. Use the new software automation to derive and validate new rapid-counting algorithms for evaluation as new inline tools for rapidly quantifying tissue stem cells specifically.

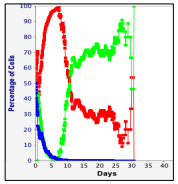
KSC Counting Technology



1. INPUT: Serial Cell Count Data



2. Computational Simulation
(Average of 10 best from 10,000 trials)



3. KSC Counting OUTPUTS
Blue – *Tissue stem cells*
Red – Committed progenitor cells
Green – Arrested differentiated cells

PROJECT COURSE

The original TORTOISE Test KSC counting software required 3 to 4 weeks of serial culture to quantify the stem cell-specific fraction of a cell preparation.

1. Developed new **RABBIT Count** software to automate the process of deriving rapid-counting algorithms (**"Rabbit algorithms"**) from TORTOISE Test KSC counting output data.

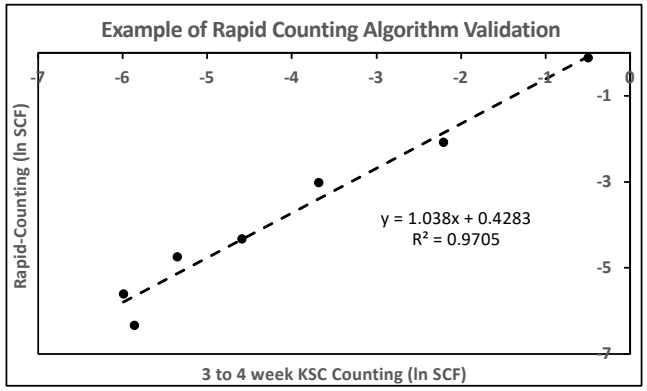
2. Verified the new RABBIT Count software automation using pre-existing TORTOISE Test KSC counting output datasets.

3. Derived new TORTOISE Test output datasets with tissue cell preparations supplied by academic labs, academic GMP cell biomanufacturing facilities, commercial stem cell product suppliers, and ARMI|BiofabUSA cell biomanufacturing development teams, including:

- University of Michigan School of Dentistry, Laboratory of Professor Darnell Kaigler
- University of Kansas, Midwest Stem Cell Therapy Center, GMP Cell Production Facility, Director Rupal Soder
- HemaCare-Charles River Laboratories
- Stemcell Technologies
- AllCells

Rapid-Counting Algorithm Validation Strategy

Compared the tissue stem cell-specific fractions (**SCF**) of samples determined by the complete 3 to 4 week KSC counting analysis to the SCF determined with rapid-counting algorithms using only 72-hour cell culture data.



PROJECT DELIVERABLES

New verified RABBIT Count software that automates the derivation of rapid-counting algorithms from TORTOISE Test KSC counting SCF data.

Validated rapid-counting algorithms that require only 72-hour basic cell culture count data to determine the stem cell-specific fraction of a biomanufacturing process sample or product sample.

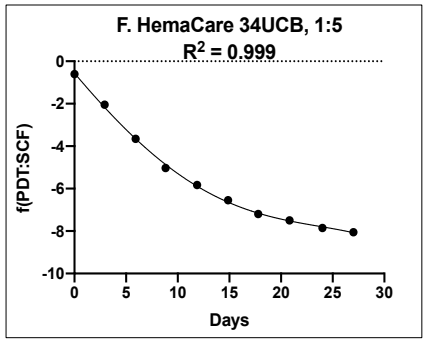
Rapid-Counting Algorithms Developed

- $CD34^+$ umbilical cord blood hematopoietic stem cells (**UCB-HSCs**)
- $CD34^+$ mobilized peripheral blood hematopoietic stem cells
- $CD34^+$ bone marrow-derived hematopoietic stem cells
- Umbilical cord tissue mesenchymal stem cells
- Dental bone-derived mesenchymal stem cells

Example of a Rapid-Counting Algorithm

Source: Commercial HemaCare-CRL $CD34^+$ UCB-HSCs

Input: 72-hour cell culture count data.
Output: Specific HSC fraction for any day of culture of the sample.



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2. 2019 Methods for Determining the Effect of an Agent on Tissue Stem Cells – GB 2529921
3. 2020 Dutton *et al.*, *OBM Transplantation*, 4(3):24; doi10.21926/obm.transplant.2003117
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