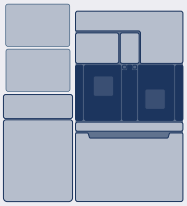
CELL THERAPY

THINKCYTE

Label-free Identification of Proliferative hMSCs



About VisionSort

ThinkCyte's VisionSort platform combines the strengths of conventional flow cytometry fluorescence signals with a novel morphometric cellular analysis measure. The dual-mode analytical capability can be used to identify and sort phenotypically defined cell populations, label free, using machine learning approaches.

INTRODUCTION

The use of human mesenchymal stem cell (hMSCs)-based therapies is a promising new approach for the treatment of various diseases due to their widespread availability, relative ease to culture and expand in vitro, ability to differentiate into several different cell types, and their limited immunogenicity. Development of effective hMSC-based cellular therapies requires non-destructive assessment of phenotypes that are indicators of expansion and therapeutic potential. One such phenotype is cell proliferation, as the expansion of hMSCs to therapeutic quantities in vitro requires highly proliferative starting cell populations. Since cell proliferation can be reflected in cell morphology, we assessed the ability to use morphological profiling with VisionSort to identify and isolate hMSCs with higher proliferative capacity as an upstream step in the development of hMSC-based cell therapies. We show that using VisionSort, hMSCs with specific proliferative phenotypes can be isolated label-free and used in R&D efforts to optimize the production of cell therapy products.

RESULTS

Frozen normal bone marrow derived hMSCs were cultured for four weeks and stained with CarboxyFluoroscein Succinimidyl Ester (CFSE), a fluorescent reporter dye that is used as a surrogate for assessing cell proliferation rates (cells with higher proliferation rates show lower CFSE intensity levels due to dilution of the dye during cell division). A machine-learning derived classifier model for cell proliferation rate was trained on VisionSort using CFSE-stained hMSCs and supervised learning (**Figure 1**). The classifier model was developed to discriminate between rapidly proliferating cells (those in the lower 20% of all cells by CFSE intensity) and more slowly proliferating cells (those in the upper 20% of all cells by CFSE intensity).

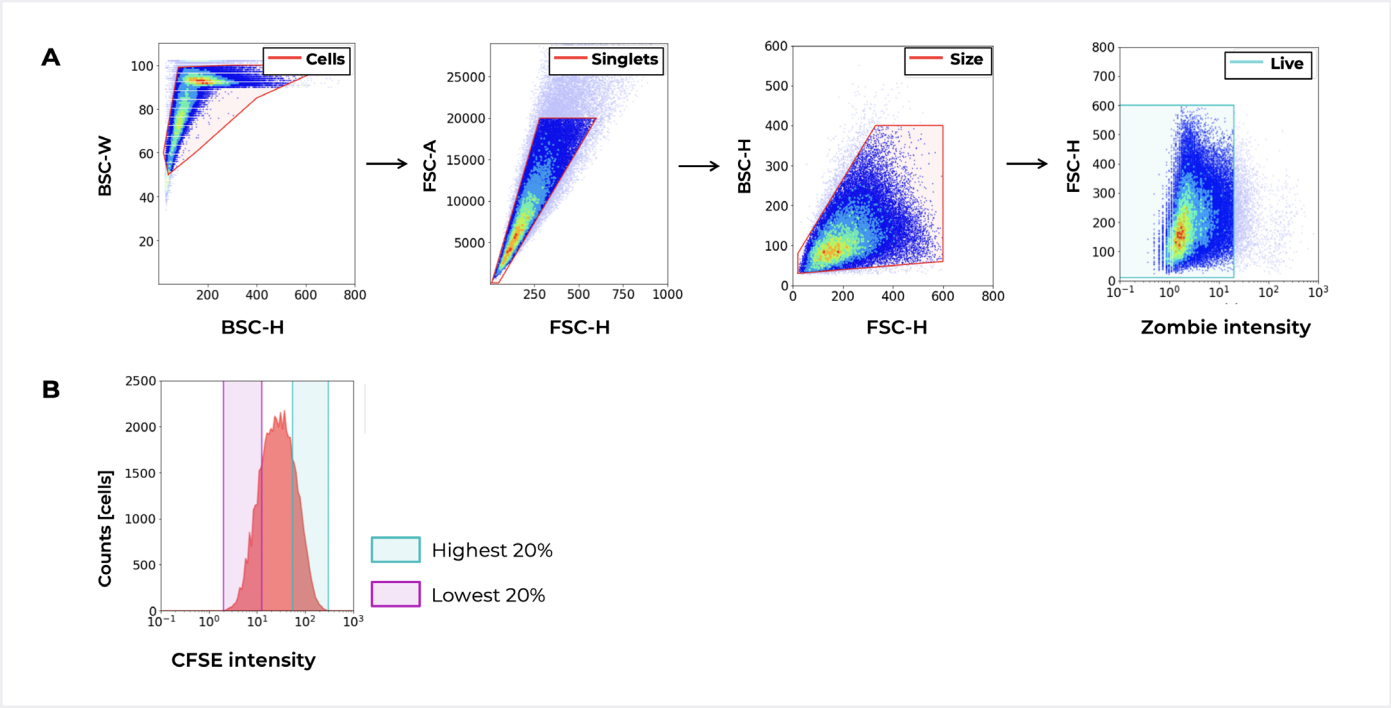


Figure 1. Gating strategy used to develop the proliferation classifier model by supervised machine learning. Agating hierarchy **(A)** was used as an initial strategy to identify live cell singlet event populations. From this, cells were gated based on CFSE fluorescence intensity **(B)** to define the faster proliferation (lowest 20% of CFSE intensity, magenta) and slower proliferation (highest 20% of CFSE intensity, aqua) populations used to train the machine learning classifier model for proliferation phenotype.

The classifier yielded a ROC-AUC score of 0.971 +/- 0.002, indicating great performance for discriminating between proliferation phenotypes (**Figure 2A**). When label-free morphological profiles from individual cells were analyzed by unsupervised machine learning on VisionSort and visualized by Uniform Manifold Approximation and Projection (UMAP), we identified two distinct clusters separating the rapidly and more slowly proliferating populations (**Figure 2B**). We confirmed that cells from each population identified using high-resolution morphological profiling and unsupervised machine learning on VisionSort have substantially different CFSE staining, but were not visually separable by gross morphology using bright field images (**Figure 2C**).

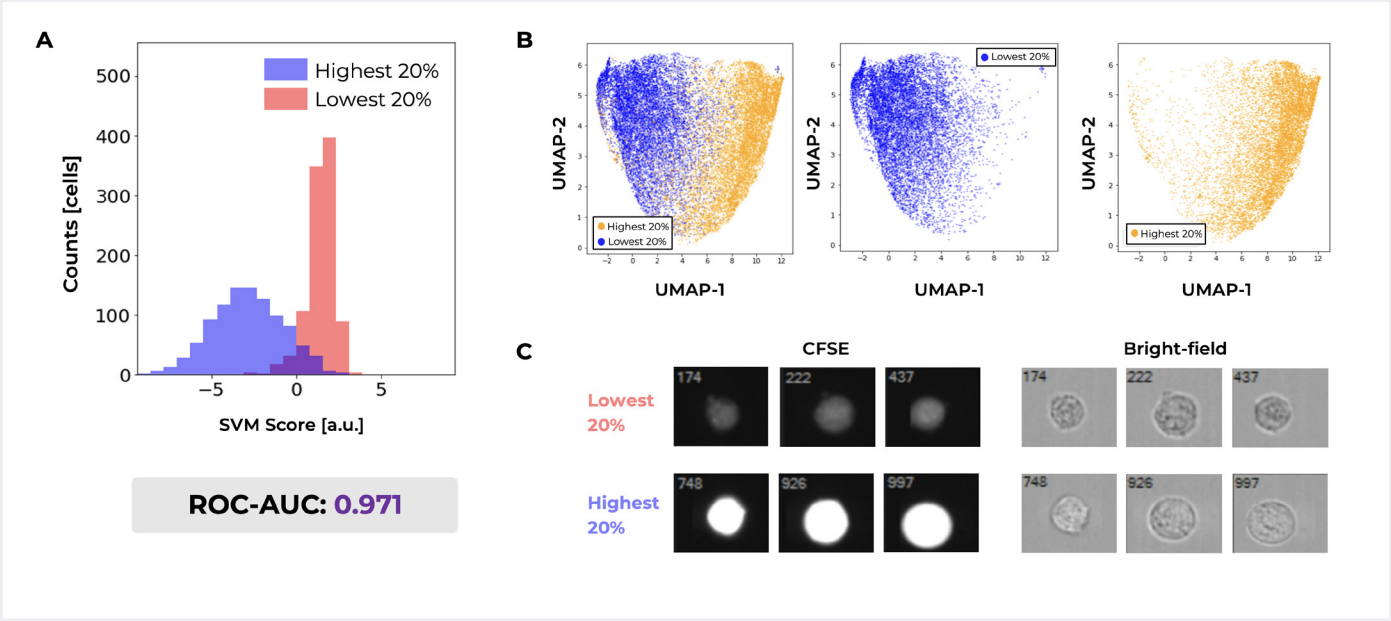


Figure 2. Generation and validation of the label-free machine learning model for proliferation phenotype in hMSCs. The proliferation phenotype classifier model showed excellent performance **(A)** when evaluated on a test cell population with an ROC-AUC of 0.971 (+/- 0.002). When morphological profiles of individuals cells were analyzed by unsupervised machine learning and visualized by UMAP, rapidly proliferating (lowest 20% of CFSE staining, blue) and more slowly proliferating cells (highest 20% of CFSE staining, yellow) were clearly distinguishable **(B)**. Single cell images (taken using an imaging cytometer) showed clear differences in CFSE staining between rapidly proliferating (lowest 20% of CFSE staining, red) and more slowly proliferating cells (highest 20% of CFSE staining, purple) while gross morphology by bright-field imaging was similar **(C)**.

SUMMARY

Selecting optimal cells for use in hMSC-based therapies requires the non-destructive assessment of production-capacity related phenotypes, such as cell proliferation. Here we show that morphometric profiling using VisionSort can characterize and identify hSMCs based on proliferation rates, label-free. The approach described here can be used to isolate rapidly proliferating cells, untouched by external markers or labels, for early cell therapy development workflows.

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