A rapid image cytometry method for quantification of canine stromal vascular fraction cells

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1. ABSTRACT

In recent years, the lipoaspirate collected from adipose tissue has been seen as a valuable source of adipose-derived mesenchymal stem cells for autologous cellular therapy. For multiple applications, adipose-derived mesenchymal stem cells are isolated from the stromal vascular fraction (SVF) of adipose tissue. Because the fresh stromal vascular fraction typically contains a heterogeneous mixture of cells, determining cell concentration and viability is a crucial step in preparing fraction samples for downstream processing. Due to a large amount of cellular debris contained in the SVF sample, as well as counting irregularities standard manual counting can lead to inconsistent results. Advancements in imaging and optics technologies have significantly improved the image-based cytometric analysis method. In addition, fluorescence detection using novel fluorescent probes have improved sensitivity of detection methods. In this work, we validated the use of fluorescence-based image cytometry, Cellometer Vision, for SVF concentration and viability measurement. We compared the Cellometer Vision to the current methods, standard flow cytometry and manual hemocytometer. Five freshly collected canine SVF were analyzed using all three methods to measure concentration and viability. The results were highly comparable, which validated the image cytometry method for canine SVF analysis, and potentially for SVF from other species.

2. CELLOMETER IMAGE CYTOMETRY FOR SVF ANALYSIS

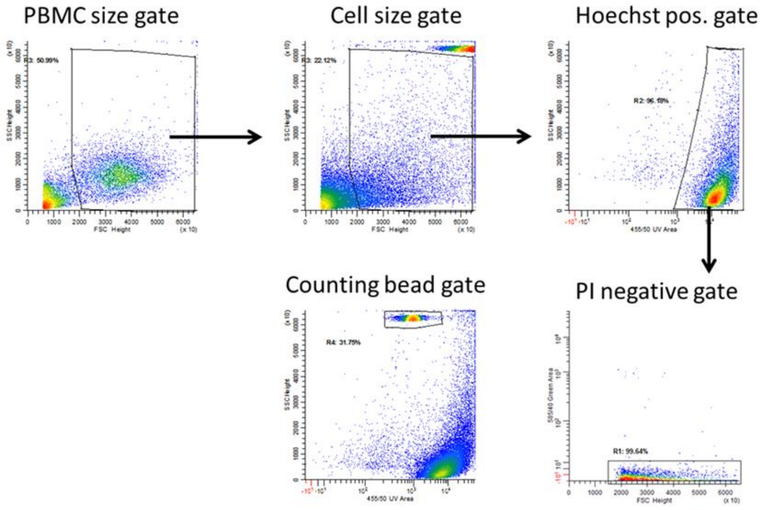
Results:

Count	Concentration
Total: 1035	1.16x10^7 cells/mL
Live: 954	1.07x10^7 cells/mL
Dead: 81	9.06x10^5 cells/mL

Viability: 92.2%

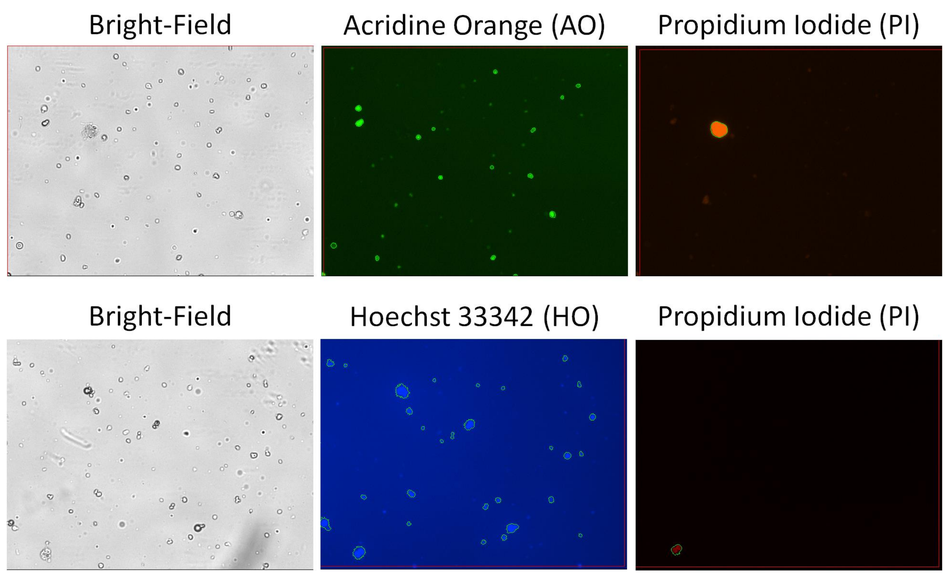
1. Cellometer Image Cytometer is a slide-based cytometer that can capture bright-field and fluorescent images in a disposable counting chamber
2. The captured images are analyzed with the Cellometer software to measure size, morphology, cell count, and fluorescent intensity
3. The measured parameters are used to generate GFP/RFP expression, DNA cell cycle analysis, apoptosis, and cell viability results

3. STANDARD FLOW CYTOMETRIC ANALYSIS OF SVF SAMPLES



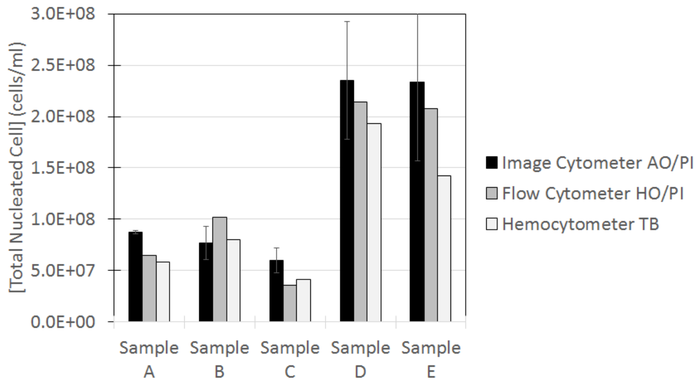
- The standard flow cytometric analysis gating scheme is shown on the left
- The gating is used to generate cell count and concentration on the flow cytometer
- Initially, canine PBMCs were used to set the initial cell size gating.
- Next, the cell size gate was transferred onto each SVF sample.
- A Hoechst fluorescence gate was set to identify nucleated SVF cells
- A propidium iodide fluorescence gate was set to determine the percent viable cells
- Finally, a gate was set on the counting beads to determine the concentration of the SVF sample
- The results from flow cytometric analysis are compared to the Cellometer results

4. CELLOMETER CAPTURED FLUORESCENT IMAGES



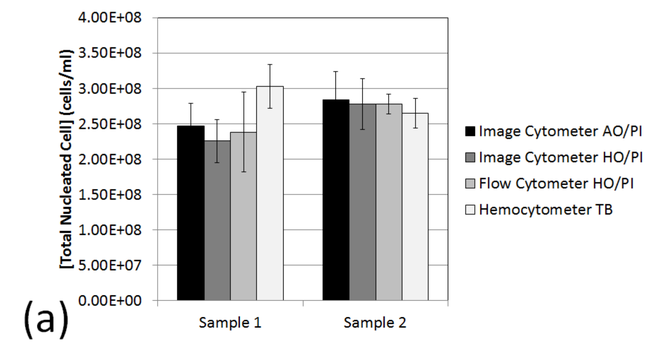
- The Cellometer Vision is used to capture bright-field and fluorescent images of SVF samples stained with AO/PI and HO/PI with pseudo color of green Acridine Orange (AO), orange Propidium Iodide (PI), and blue Hoechst (HO)
- The AO is detected by the filter VB-535-402, the PI is detected by the filter VB-660-502, and the HO is detected by the filter VB-450-302
- The bright-field images showed numerous fluorescent and non-fluorescent particles, indicating nucleated cells and cellular debris, respectively.
- By using fluorescence, the debris and nonspecific particles are not counted using Cellometer or flow cytometer

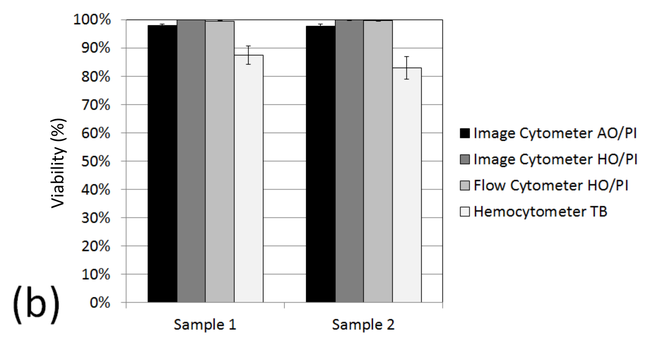
5. TOTAL CELL COUNT RESULTS COMPARISON



- Concentration comparison results of 5 SVF samples between hemocytometer, image and flow cytometry method
- The experiment was conducted to optimize imaging parameters for the image cytometry method, which showed comparable concentration analysis for Sample A-D between all three methods.
- The deviations were approximately ±10%. As for Sample E, the image and flow cytometry method showed good correlation.
- In contrast, the hemocytometer result was approximately 30% lower, which may indicate the difficulty of manual counting highly concentrated samples (Similar trend shown in Sample D, where hemocytometer result was lower)

6. CELL CONCENTRATION AND VIABILITY COMPARISON





- Concentration and viability comparison results between hemocytometer, image and flow cytometry method
  - (a) Concentration comparison
  - (b) viability comparison
- Using AO/PI staining on Cellometer, using HO/PI staining on both instruments, and Trypan blue on hemacytometer
- Both results showed comparable measurements between all 4 methods
- For the sample 1 concentration measurement, the hemocytometer method showed approximately 10% higher variation compared to image and flow cytometry
- This could be due to bright-field counting of debris and nonspecific particles
- For viability measurements, both samples showed approximately ~15% reduction in viability with Trypan blue
- This could be due to counting nonspecific particles as dead cells, which decreased the viability %

7. SUMMARY AND CONCLUSION

- Cellometer Vision image cytometer was validated for measuring concentration and viability of primary canine stromal vascular fractions
- These cells are extremely difficult to count using manual hemacytometer and trypan blue method due to the amount of debris and nonspecific particles present in the sample
- By utilizing fluorescent staining such as Acridine Orange, Propidium Iodide, and Hoechst 33342, the nucleated cells can be stained specifically and eliminate the error from counting the debris
- The flow cytometry and Cellometer counting method showed comparable results, which validated the Cellometer image cytometry method

Reference

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