Determination of contaminating particles in cell therapy products; a proof of principle study.

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Introduction

Distinguishing between different subvisible particles (SVP) species within a cell therapy product is not facile and has proven challenging using standard techniques such as flow cytometry[1]. This is, in part, due to the cell therapy product contributing to the SVP population.

This has recently been exemplified by the recent FDA issuance of a Form 483 concerning particle contamination of the Car-T cell therapy Kymriah® with wood, cellulose, brass, and steel[2]. It is generally considered to be attributed to the cryopreservation bags. This recent observation has highlighted a need for a new approach to determining SVPs within cell therapies.

Using a novel technique, we employed backgrounded membrane imaging (BMI), side illumination membrane imaging (SIMI), and fluorescence membrane microscopy (FMM), available on the Aura+, to examine the presence of plastic and other extrinsic particles in a model Car-T cell population.

This proof of principle study aimed to determine the viability of extrinsic particle analysis using Aura+ in complex cell therapies.

BMI and SIMI Explained

Briefly, subvisible particles were immobilized on a backgrounded 0.8µm porous filter membrane using vacuum (15 inHg) and imaged using epi-illumination (BMI) and side-angle illumination (SIMI) to determine all particles and extrinsic particles, respectively. Extrinsic particle remain rigid and thus exhibit a positive SIMI signature (see figure 1).

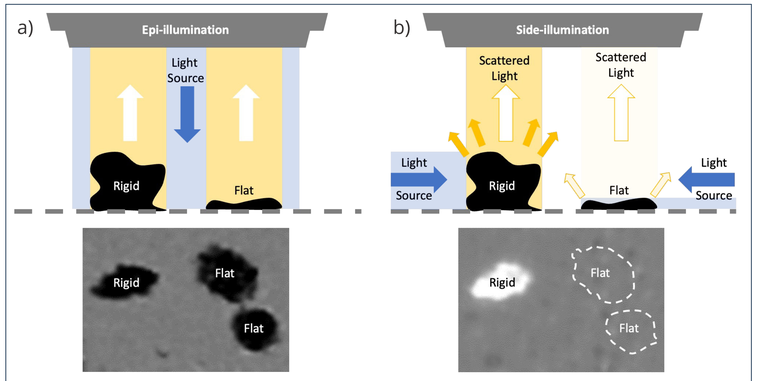

Figure 1. Schematic representation of BMI and SIMI.
a) BMI utilizes epi-illumination to capture all particles in high contrast; b) SIMI can distinguish rigid extrinsic particles from flat biologic particles.

Extrinsic Particle Counting

To model the presence of extrinsic SVP in cell therapies, Car T cells were mixed with the NIST particle standard (abraded Ethylene tetrafluoroethylene)[3], cellulose fibers suspended in filtered water for injection (WFI), and metallic particles (commercially available iron fillings suspended in filtered WFI). To assess particle populations, 50µL of each sample was loaded in triplicate onto a backgrounded filter membrane (0.8 µm pore size) and analyzed. Herein, a mean extrinsic particle count of 1.1x10^4/mL (SD±0.05), 2.9x10^4/mL (SD SD±0.15), and 0.5x104/mL (SD±0.05) respectively (see figure 2).

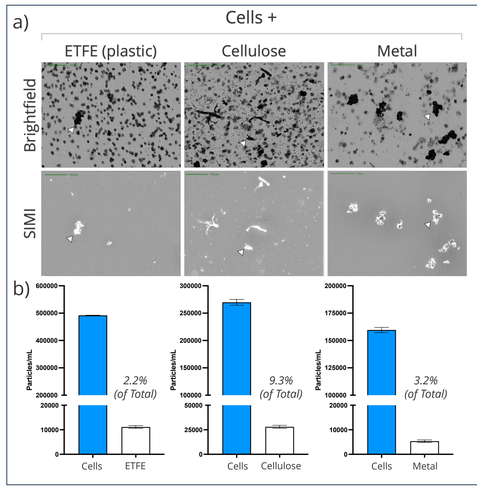

Figure 2. Extrinsic Particle Counting using Aura+.
(a) Car T cells mixed with either plastic (ETFE), tissue or metal SVPs were imaged using BMI (brightfield) and SIMI. In all three images extrinsic particles (▵) are identified as white particles with a SIMI intensity >10; biologic particles (cells and protein) are only visible in the brightfield channel.
(b) Extrinsic particle counts were determined based on an equivalent circular diameter (≥5um) and SIMI intensity (>10). Cells were defined as diameter (≥5um) and SIMI intensity (≤10).

Residual Dynabeads™ Detection

Identifying residual Dynabeads* in cell therapies, less than 100 per 3x10^6 cells, is an important consideration[4]. SIMI analysis can be used to count residual beads in a Car T cell preparations. Herein, we analyzed 1x10^6/mL Car-T cells mixed with CD3/CD28 activation Dynabeads (figure 3).

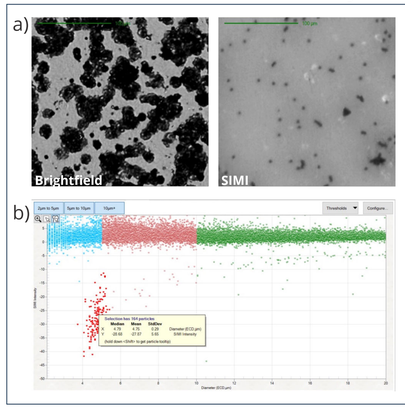

Figure 3. Residual Dynabeads Detection using SIMI mode.
(a) Brightfield analysis exhibited multiple cell clusters, however when SIMI analysis was used Dynabeads could be easily distinguished.
(b) Plotting the particle data using a 2D scatterplot Dynabeads populations can be easily selected, counted and analysed. Herein we measured singlet Dynabeads with mean diameter of 4.75 (SD ±0.29) um and mean SIMI intensity -27.87 (SD ±5.6). The LOQ was determined at 10 beads/mL (data not shown).

*Disclosure: Dynabeads refers to the magnetic beads produced by Thermo Fisher Scientific, Inc. Halo Labs is not affiliated with Thermo Fisher Scientific, Inc., and references to Dynabeads or any other third-party trademark do not imply sponsorship, endorsement, or approval.

FMM for multiplex analysis

The Aura+ incorporates three fluorescence channels, FL-1 (ex:440 em:500), FL-2 (ex:376 em:440), FL-3 (ex:482 em:524). Combined with BMI and SIMI analysis, FMM allows for total particle analysis within a complex particle cell mixture. Briefly, Car T cells (5x10^5/mL) were treated with 5x freeze-thaw cycles (to stimulate cell death), stained with DAPI (2µg/mL) to resolve nuclear details, and Thioflavin T (ThT) to proteinaceous particles. Four particle subtypes were identified; plastic (SIMI$^+$), non-viable cells (Dapi^{high}), viable cells (Dapi^{low}), and protein aggregates (ThT$^+$ Dapi$^-$) (figure 4).

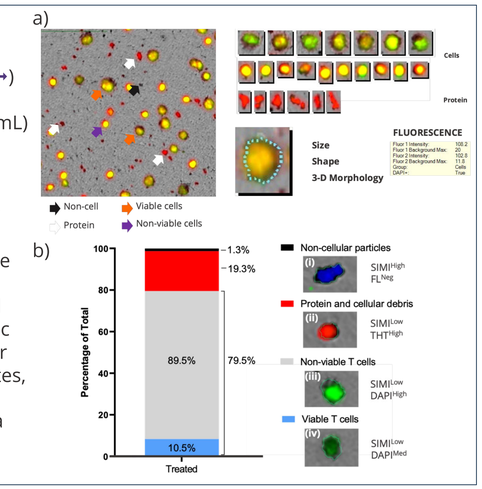

Figure 4. Multiplex Analysis using FMM on Aura+.
a) Viable (→) and non-viable (→) cell populations were mixed 50:50, stained with Dapi (5µg/mL) and Thioflavin T (5mM), and imaged. Cell populations were defined using FL-2 intensity (Dapi); 10-100 and 150-250 for viable and non-viable cells respectively. (b) Using the same staining strategy a viability assessment can be performed alongside determining extrinsic (SIMI$^+$) contaminants and other biologic particles and aggregates, providing a full picture of the particulate population within a given cell therapy.

Conclusion

This proof of principle study highlighted the capability of Aura+ to identify, count, and size different subvisible particle populations within a complex mixture of cells, protein, and plastic particles.

Thus, providing an important breakthrough in counting contaminating extrinsic particle populations within cell therapy products.

References

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