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Platelets induce regulatory T-cell expansion during CAR-T cell manufacturing

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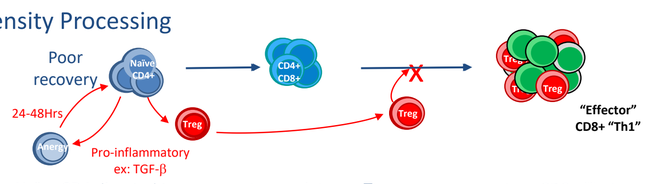
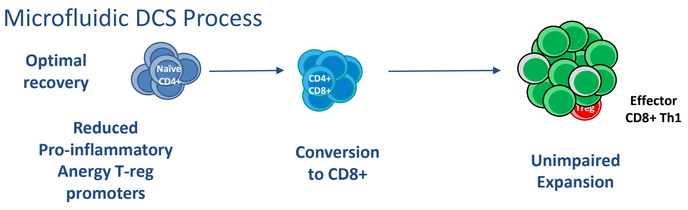
1. Abbreviated Abstract

Consistent white blood cell collection, with high recovery of Naïve T cells and their subsequent expansion into T central memory, and T stem central memory cells (Tcm, Tscm) are critical for successful manufacturing of CAR T with an optimal profile for cell therapy[1]. However, the currently used centrifugal approaches have limitations due to absolute and relative bias away from younger T cell phenotypes present in density-based approaches (1a). In addition, elevated platelet:T cell ratios were found in the density gradient based interface, which left the cells including naïve T-cells exposed to a proinflammatory milieu during processing resulting in conversion of naïve cells to a T-regulatory phenotype. In sharp contrast, use of microfluidic DCS processing (1b) with its rapid and intrinsic 3-log wash reduced the pro-inflammatory milieu by more than 5-fold for a number of proinflammatory cytokines, resulting in up to *50% less* regulatory T-cell (T-reg) generation during expansion.

1a Density Processing



1b Microfluidic DCS Process

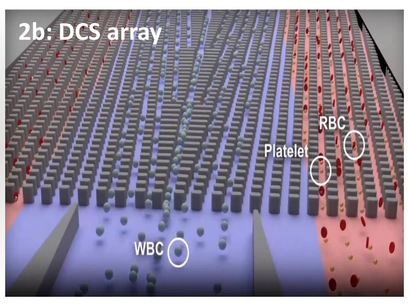


2. Deterministic Cell Separation

Deterministic Cell Separation (DCS), separates cells much like a "pachinko" or coin-sorter, which separates on the basis of size as the particle (cell) passes through an obstacle array which is sized and positionally defined such that particles that are smaller than a specific size pass though the device along a streamline (pink), and cells larger than that specific size are repeatedly guided into a clean buffer stream (blue). For cell therapies, a mononuclear (MNC) apheresis collection containing platelets, erythrocytes and leukocytes is fluidically processed through the highly parallel 3.5 million micro post array configured as shown, which is oriented to separate cells >4um. The Curate cell processing system manages and monitors all fluid movements across the array. The system and array (2a,b) is capable of processing WBC at rates of 50x10^9/Hr and depletes >99.5% of platelets and >90% of residual RBC from a leukopack while recovering WBC with viability similar to the input, all while delivering a 3-log wash efficiency.

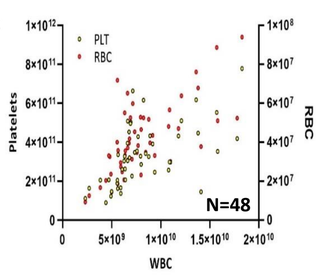
2a

2b: DCS array

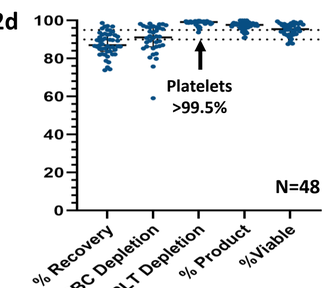


The uniform and gentle processing of the array recovers >96.5% of WBC processed (% product) in an unbiased fashion while removing >99.5% removal of platelets. Loss of aggregates to the 20um prefilter lower average recovery to ~90%. Range of Inputs evaluated in beta testing (2c) and recovery metrics (2d).

2c

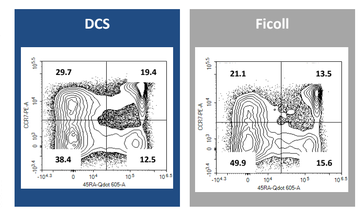


2d

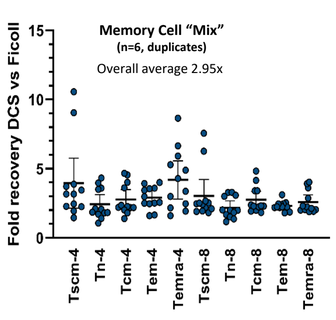


In addition to the absolute recovery advantage of DCS previously published, a bias away from rosette forming cells[2] shows a significant range of relative recovery of younger phenotypes in both Ficoll processing (2e,f) and Counterflow Elutriation, with Ammonium Chloride also showed an almost 50% loss of Naïve T cells in split samples[3,4] (2g)

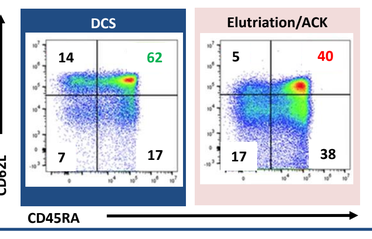
2e



2f



2g

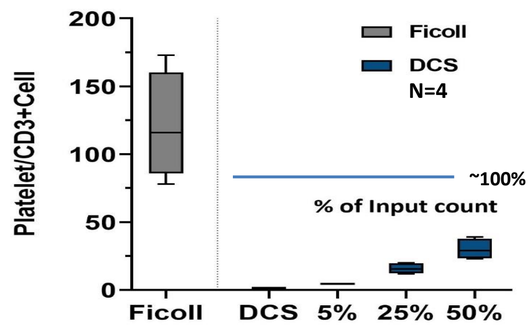


Optimal recovery of naïve T cells is important for optimal CAR-T profile

3. Comparison of DCS, Ficoll Washes and T-reg generation

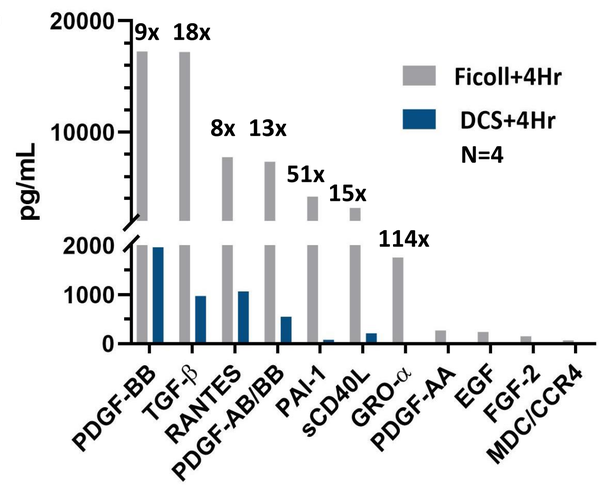
Our Previous work[5] showed beneficial expansion of T-central memory expansion when DCS was used. Using manufacturers standard wash recommendations in apheresis sample the concentration of platelets/aggregates at the Ficoll interface resulted in >1.5x autologous platelet:CD3 ratio during Ficoll processing, and DCS "add back" experiments (3a).

3a



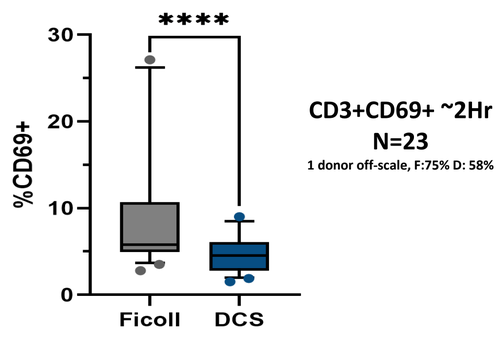
Evaluation of 52 plex cytokine/chemokine abundance 4 Hrs post separation with 2 washes - shows multiple residual proinflammatory cytokines/chemokines >5 fold higher and at >50pg/mL levels (3b):

3b



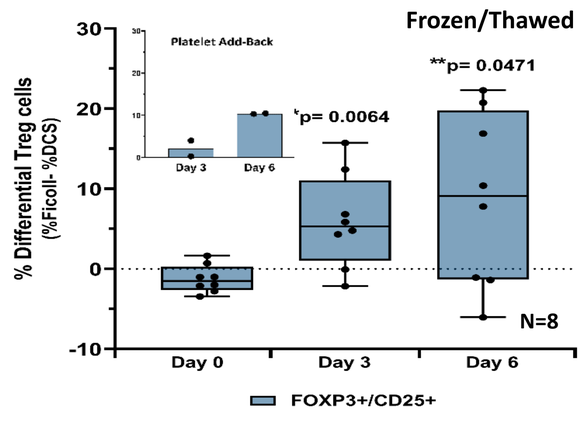
DCS cells show ~50% less CD69 upregulation on CD3 cells post processing (3c).

3c



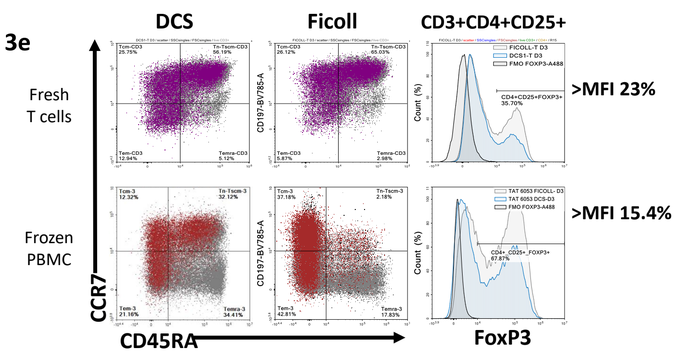
Definitive differential identification of CD3+CD4+25+ and FoxP3+ve T regulatory cells and platelet spiking control (inset, using fresh cells) and evaluated on frozen/thawed PBMC activated via CD3/28 activation (Transact) and 5ng/mL of IL-7 and IL-15 in TexMACS media. (3d)

3d



Example dot plots of Fresh isolated CD3+ or Frozen CD3+ PBMC for T memory subsets measured at day 3 following CD3/28 activation as in 3d. T regulatory cells are back-gated from FoxP3 3e.

3e

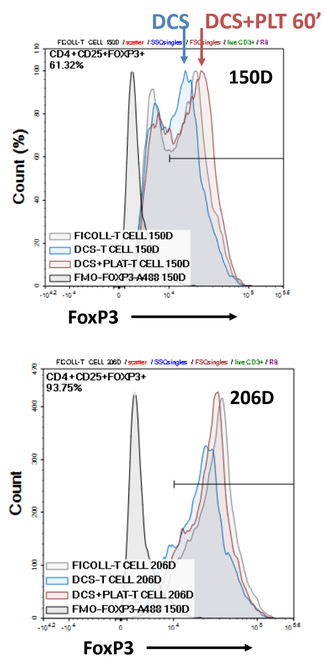


3. Continued

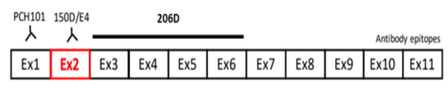
1x Autologous platelet milieu add back to fresh PBMC causes up regulation of both immunosuppressive (Exon 2, clone 150D), and pan FoxP3 (clone 206D) in DCS prepared cells. (3f) Representative data shown.

3f

1 Hr exposure to platelets increases FoxP3 median fluorescence stain intensity by ~23%

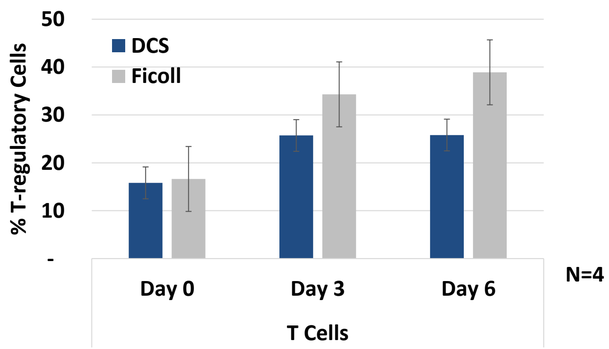


150D/Exon 2 responsible for immunosuppressive effects



Confirmation of continued T cell expansion and composition of T-regulatory cells in same culture conditions as in 3d, (3g).

3g



4. Conclusions

1. **DCS processes apheresis and recovers leucocytes without bias and without exposure to density matrix or Ammonium Chloride.**
2. **Rapid and efficient removal of platelets and soluble factors using microfluidics has a material (beneficial) impact on the biological phenotype of the cells when expanded.**
3. **DCS shows >5 fold reduction in the level of multiple key pro-inflammatory cytokines ahead of cell engineering, and show ~50% less CD69 expression.**
4. **Elevated Fox-P3/T reg expansion preferentially occurs in both fresh and frozen preparations of PBMC and isolated T cells by density gradient methodology. DCS prepared cells show approximately 50% of the amount of regulatory T cells vs. Ficoll.**
5. **Add back of platelet milieu to DCS prepared cells causes the upregulation of the immunosuppressive Exon 2 and is detectable within 60 minutes (% and MFI increase).**
6. **Overall, DCS prepared cells yield a more optimal starting material suitable for T-cell therapy manufacturing.**

5. Key References

1. Good, Z. et al. Nat Med 28, 1860–1871 (2022).
2. Hokland P. et al. Scand J. Imm. 1980, 11(3):353-356
3. DCS White Paper www.curatebio.com/publications
4. Plank K, et al., Int J Lab Hematol. 2021;43(5):939-947.
5. Campos-González, et al., 2018. SLAS Technology: 23(2)

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