Engineered Textiles as Substrates for Cell Selection and Activation

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Purpose: T cell-based therapies utilize T cells intrinsic binding ability towards antigens present on the surface of cancer cells to offer a more immune-friendly treatment option for patients compared to traditional chemotherapies. Production of such cell therapy products is reliant on efficient generation of clinically sufficient quantity of cells from limited patient donor cell supply. Effective cell selection using cell-specific surface markers is critical for obtaining specific cell subpopulations from patient apheresis material for further processing. T-cell activation is a key event in the adaptive immune response and is vital to the expansion step of T cell therapy production. This study was focused on use of engineered polyethylene terephthalate (PET) textiles with surface immobilized antibodies to select subpopulations of cells and then activate them. Improvements in cell therapy product quality and quantity can help overcome the challenge of targeting of antigens on tumors which remains a major obstacle in current cell therapy research[1]. Several PET textile structures, having differing weave patterns and filament types, were investigated to identify structures that demonstrated the best capacity to select and activate cells.

Methods: Textiles, sized to fit 96 well plates, were ultrasonically cleaned with a 70% IPA solution, dried, and then incubated in 0.5N NaOH for 24 hrs to activate surface functional groups. The activated textiles were then rinsed with MES buffer, and then incubated for 30 min in 0.5 g/ml EDC and NHS solution on an orbital shaker Textiles were rinsed with PBS and then were incubated for 2 hours at RT with either 300 µl of 40 µg/ml anti-CD19 antibody solution, or a mixture of anti-CD3 + anti-CD28 antibodies, to immobilize the antibody to the PET textile surface.

For Toledo cell *selection experiments*, a cell suspension mixture was prepared having 4.0E+5 cells/sample at a 1:1 Jurkat E6-1 cell (ATCC #TIB-152) to Toledo cell (ATCC #CRL-2631) ratio. 200 µl of the mixed cell suspension was incubated with each sample for 2 hours at 37°C, 5% CO_2 to preferentially select out the Toledo cells. Textiles were rinsed twice with PBS to remove any non-selected cells from the surface following incubation. Selected cells were then stained with 100 µl of Hoechst stain diluted 1:2000 in PBS. Images were taken to visualize cell attachment to the textile surface using an EVOS M7000 fluorescence microscope (ThermoFisher, Waltham, MA). Non-selected cells were counted, fixed, and then were labeled for CD3 and CD19 (BD Biosciences, Franklin Lakes, NJ) plus DRAQ5 nuclear stain (Invitrogen, Carlsbad, CA) for flow cytometry using a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA) to determine the cell populations after selection.

Jurkat cell *activation studies* were characterized through the presence of CD69 on the cell surface following 18 hours exposure of 2.0E+5 Jurkat cells/sample to anti-CD3/anti-CD28 PET textiles. For flow analysis, fixed cells were labeled for CD69 (BD Biosciences, Franklin Lakes, NJ) and DRAQ5 show the percent shift of $CD69^+$ cells. Activation was confirmed through measurement of IL-2 and TNFα levels using Lumit™ cytokine detection kits (Promega, Madison, WI) pre- and post-activation.

Results: The multi-filament weave structure exhibited the highest degree of selection at 57.1% Toledo cells selected.

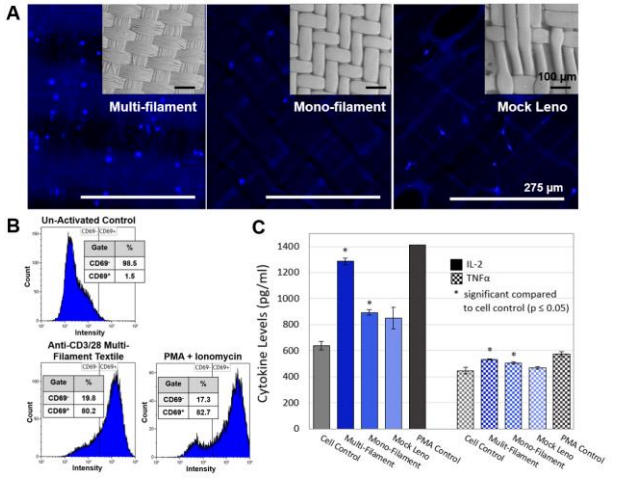

Figure 1: (A) Impact of textile structure on Toledo cell selection, (B) Increase of $CD69^+$ Jurkat cells following activation using textile, (C) cytokine increase upon activation with several textile structures.

The multi-filament textile also showed the highest degree of cell activation with an average $CD69^+$ shift of 80%. This $CD69^+$ shift is comparable to pharmacological activation reagent, phorbol myristate acetate (PMA) + ionomycin, which averaged a $CD69^+$ shift of 83%, and has been known to show $CD69^+$ shifts of up to 95% in other studies[2]. Both multi- and mono-filament textiles showed an increase in IL-2 at 650 pg/ml and 235 pg/ml average increase respectively relative to un-activated cells. Similar relative cytokine increases were seen with TNFα levels. Multi-filament textile cytokine increases were slightly below the PMA + ionomycin control, consistent with the slightly lower peak intensity seen with the CD69 data. Given these results, the higher surface area of the multi-filament textile has highest potential to improve efficacy of cell selection and activation for generation of cell therapy products.

References:

[1] (Sterner, R.C. *Blood Cancer Journal.* 2021;11:69)
[2] (Andersen, N. *Clin. Diag. Lab. Immu.* 2002;9:243-250)

Disclosure Statement: All work presented herein has been performed by employees of Secant Group, LLC.