FUNCTIONALIZED MICROCARRIERS FOR ENHANCED CAR T CELL MANUFACTURING

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Adoptive cell therapy using chimeric antigen receptor (CAR) T cells has shown immense promise in treating cancer. In 2017, Novartis and Kite Pharma both acquired FDA approval for their CAR T cell therapies targeted toward B cell malignancies. Despite these successes, manufacturing high-quality T cells at scale has proven challenging. Notably, current processes rely on bead-based expansion and suspension-cell bioreactors, which do not recapitulate the high cell density and robust signaling found in the lymph nodes.

We hypothesized that recreating the human lymph node using functionalized biomaterials could lead to increased T cell expansion. This strategy involved functionalizing macroporous, gelatin microcarriers (commonly used throughout the bioprocess industry) with CD3/CD28 antibodies to provide activation signals. Cells can adhere to these antibodies and grow along the surface and throughout the interior, enabling efficient auto/paracrine signaling. Gelatin, as a collagen derivative, also provides matrix signals.

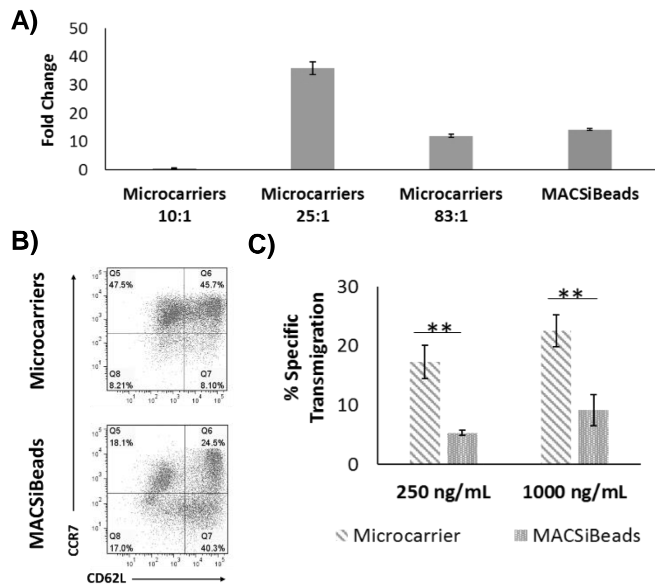

Figure 1 - a) fold change after 14 days b) surface expression of CCR7/CD62L c) chemotaxis transwell assay

Primary human T cells were incubated with carriers or conventional magnetic activation beads. Carriers cultures showed a 3-fold increase at the 25:1 ratio as compared to bead cultures (fig 1a), indicating that microcarriers can provide superior expansion if optimized for culture conditions. Furthermore, the phenotype of microcarrier-expanded T cells had a greater frequency of CD62L+CCR7+ T cells than beads, indicating higher frequency of memory T cells (fig 1b). This trend held true across multiple experiments and donors, with carriers producing 11% more memory cells than average. The functionality of memory T cells was confirmed using a CCL21 transwell assay (fig 1c). We also transfected T cells with an anti-CD19 CAR lentivirus and confirmed anti-tumor functionality after 14 days of expansion using a CD107a degranulation assay.

We further hypothesized that the microcarrier culture could be optimized using design of experiments (DOE) methodology to interrogate meaningful process parameters. The effect of IL2 concentration, initial cell density, media type, activation signal strength, and microcarrier concentration on the resulting fold change and memory phenotype were investigated. Linear regression showed that all factors except initial cell density had a significant effect on fold change, indicating that our process is not dependent on obtaining a fixed number of cells from patients. Furthermore, we found that only IL2 had an effect on memory phenotype, where an optimum was found at an intermediate setting. In summary, this information is useful in industrial settings where the concern is producing high quality cells with minimal resources.

We have demonstrated that microcarriers can provide robust expansion of high-quality memory T cells compared to state-of-the-art methods while maintaining anti-tumor functionality. Furthermore, we demonstrated that DOE methodology can be meaningfully applied to determine optimal process parameters. Further studies will be conducted in scalable bioreactors to develop translatable processes for this technology.