Rapid Manufacturing of Monocyte-Derived Dendritic Cells (MoDC) in a GREX Device with a Seamless Transition to the Production of Tumor-Associated Antigen-Specific T Cells (TAAT) in the Same Device

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Background & Aim

MoDC can be used as cancer vaccines or antigen presenting cells for the generation of antigen-specific T cells. Traditional MoDC manufacturing is labor-intensive; the adherent nature of the cells requires physical or chemical manipulation, which leads to cell loss and impaired function. The open process must be conducted in a GMP space and is not feasible for institutions without clean room capabilities. Automated MoDC manufacturing systems exist but are not available in most labs. We developed MoDC manufacturing protocol in a GREX device – a closed-system GMP-amenable culture vessel with a nonadherent gas-permeable membrane at the base, which reduces cell loss, and allows seamless transition to downstream applications.

Methods

Monocytes were isolated from 30 mL of peripheral blood by CD14 immunomagnetic selection, seeded in GREX at 0.25 – 0.5e6 cells/cm^2, differentiated x3-5 days into immature DC with GM-CSF/IL-4, loaded with peptides (PRAME, Survivin, WT1), and matured x24-48 hours with TNF-a/IL-6/IL-1b/PGE2 (n = 7). MoDC manufactured without antigen loading served as a negative control. MoDC function was assessed by priming donor PBMC with antigen-loaded autologous MoDC, expanding x9-14 days, and rechallenging with antigen-loaded MoDC. A 7-day method (n = 2) was compared with a rapid 4-day protocol (n = 5). TAAT expansion trends were analyzed to explore the potential of reaching sufficient cell numbers for clinical dosing (0.5e4 cells/kg).

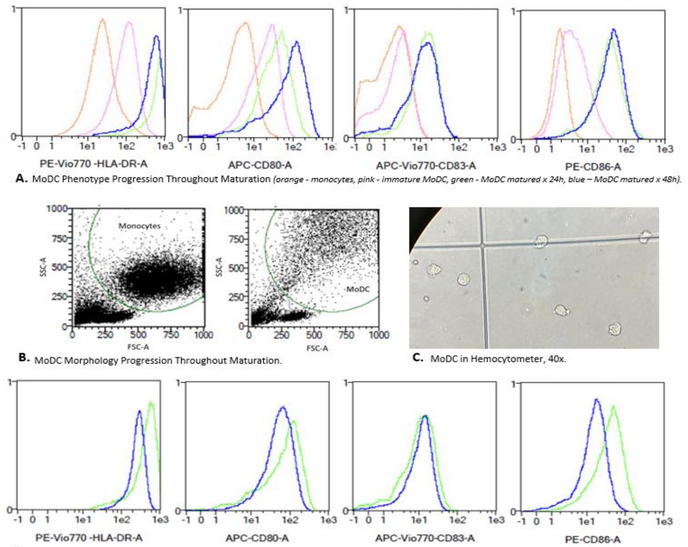
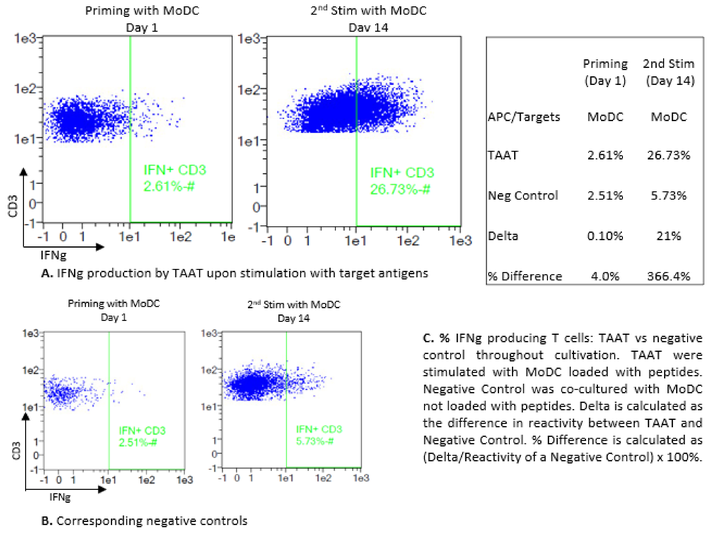


Figure 1. A. MoDC Phenotype Progression Throughout 7-Day Maturation (orange: monocytes, pink: immature MoDC, green: MoDC after 24 hours of maturation, blue: MoDC after 48 hours of maturation). B. MoDC Morphology Progression Throughout Maturation. C. MoDC in Hemocytometer, 40x. D. 4-Day vs 7-Day MoDC Phenotype (blue: mature 4-day MoDC, green: mature 7-day DC).



	Priming (Day 1)	2nd Stim (Day 14)
APC/Targets	MoDC	MoDC
TAAT	2.61%	26.73%
Neg Control	2.51%	5.73%
Delta	0.10%	21%
% Difference	4.0%	366.4%

C. % IFNg producing T cells: TAAT vs negative control throughout cultivation. TAAT were stimulated with MoDC loaded with peptides. Negative Control was co-cultured with MoDC not loaded with peptides. Delta is calculated as the difference in reactivity between TAAT and Negative Control. % Difference is calculated as (Delta/Reactivity of a Negative Control) x 100%.

Figure 2. A. IFNg production by TAAT upon stimulation with target antigens (representative donor). B. Corresponding negative controls. C. IFNg production by TAAT vs negative control throughout cultivation.

Results

Monocyte isolation purity was 91.8% (SD 1.5), efficiency 29% (SD 10.4)). 4 and 7-day monos showed similar increase in maturation markers HLA-DR, CD80, CD83 and CD86. Prolonged culturing was associated with higher cell loss: 4-day yield was 48.3% of starting monos (SD 8.8), 7-day - 30.0% (SD 6.0). Immature MoDC density adjusted to ~0.13x10^6 cells/cm^2 (SD 0.03) regardless of the initial monocyte seeding density. PBMC priming with antigen-loaded MoDC elicited IFNg response in 2.25% of T cells (SD 0.57; neg control 1.88%, SD 0.63) and resulted in 11.33-fold expansion (SD 7.85). 30.45% of antigen-stimulated T cells were IFNg+ upon Day 14 restimulation (SD 5.16), comparing to 13.8% neg control (SD 5.73). A 30 mL peripheral blood draw allowed to generate 50.83e6 of TAAT in 2 weeks (SD 5.14e6) – enough for multiple doses in an adult-sized patient.

Conclusions

MoDC manufacturing in GREX is a fast, effective process, requires minimal manual manipulation, is cGMP compatible, and allows streamlined transition to downstream applications.

Disclosures

None