Alginate hydrogel-based 3D fiber culture platform enables feasible and scalable human CD3 T cell production via CellFiber® technology

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Introduction

Advanced cell therapies (ACT) have shown significant clinical efficacy for cancer and autoimmune diseases using immune cells such as T, NK, and Treg cells. However, the rising demand for these therapies necessitates a scalable, feasible, and cost-efficient platform for industrial-scale manufacturing. While the GMP compliant CellFiber® platform has been successfully utilized for mesenchymal stem cell (MSC) production, its application in robust T cell manufacturing remains a key area of development.

Methods

In this study, we established a 3D fiber cell culture platform using alginate hydrogel via CellFiber® technology to produce human CD3$^+$ T cells. T cells were encapsulated using the CellFiber hydrogel fabrication system and cultured according to standard protocols using anti-CD2/3/28 activation reagents. We compared the expansion kinetics, viability, and phenotypic characteristics of these cells against conventional 2D culture systems.

Results

CD3$^+$ T cells cultured within the 3D fiber platform consistently demonstrated high viability (>95%). Notably, the fiber culture achieved a significantly greater cell expansion, exceeding 750-fold compared to conventional 2D methods (~400-fold). Phenotypic analysis revealed that fiber-cultured T cells expressed significantly higher levels of CD69$^+$ and comparable low levels of PD-1 to 2D cultures. Furthermore, the CD4/CD8 ratio kinetics and the maintenance of effector memory phenotypes remained comparable to 2D cultures, suggesting that the 3D environment promotes massive expansion without compromising essential T cell characteristics.

Conclusion

This proof-of-concept study demonstrates that CellFiber® technology provides a robust, operation friendly, and scalable solution for CD3 T cell manufacturing. This platform represents a significant step toward achieving GMP-grade, high-volume therapeutic T cell production to meet growing clinical needs.

CellFiber
Cell Culture Reimagined

(Booth #1474)

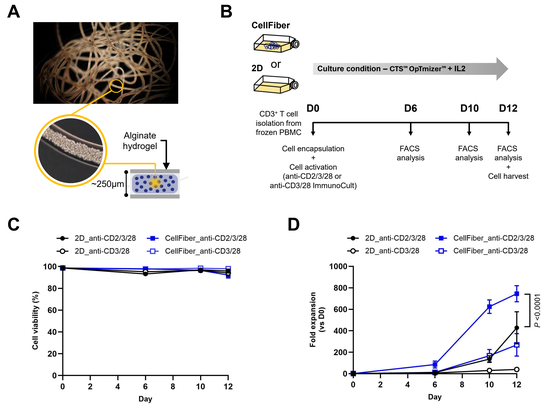

Figure 1 Human CD3$^+$ T cells show significant cell expansion after alginate hydrogel-based 3D fiber culture by CellFiber technology
A) The representative image and illustrated picture of cultured cells in gas-permeable alginate hydrogel-based 3D culture environment by CellFiber technology. B) The study design & culture schedule of CD3$^+$ T cell exapnsion in either 2D condition or CellFiber condition. Human CD3$^+$ T cells were encapsulated via CellFiber device after CD3$^+$ T cell isolation from commercially available frozen PBMCs.. For cell activation, either anti-CD2/3/28 or anti-CD3/28 activator was added in culture on Day 0. Cells were cultured in flask with culture media & IL2 for both 2D and CellFiber conditions, then cells were collected for cell count and FACS analysis at the indicated time points. C & D) Cell viability and cell expansion results were collected at indicated time points by NC-200. All data points were collected from 6 independent donors and statistical results were analyzed via 2-way ANOVA.

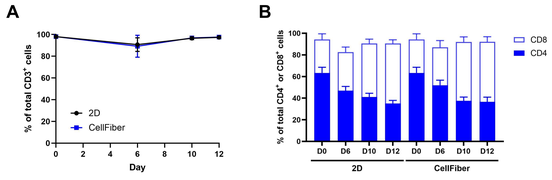

Figure 2 Fiber culture performs consistent % of CD3$^+$ T cells and comparable % of CD4$^+$/CD8$^+$ T cell kinetics to 2D culture
A & B) % of CD3$^+$, CD4$^+$ or CD8$^+$ T cells from both fiber culture and 2D culture were analyzed by FACS. Cells were collected from anti-CD2/3/28 activator condition at indicated time points. All results were analyzed by 3 independent donors.

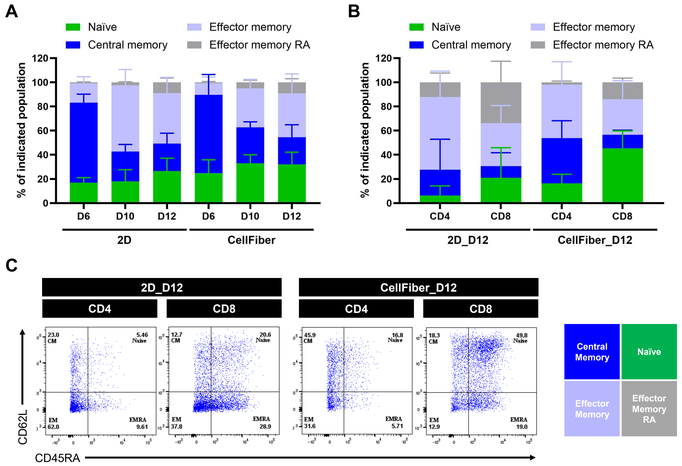

Figure 3 Fiber culture shows higher % of CD4$^+$ central memory and CD8$^+$ naïve populations after cell expansion
A) % of naïve (CD45RA$^+$/CD62L$^+$), central memory (CD45RA$^-$/CD62L$^+$), effector memory (CD45RA$^-$/CD62L$^-$) and effector memory RA (CD45RA$^+$/CD62L$^-$) T cells (total CD3$^+$ population) from either fiber culture or 2D culture were analyzed by FACS. Cells were collected from anti-CD2/3/28 activator condition at indicated time points. B) D12 samples were collected and analyzed by FACS to identify % of naïve, central memory, effector memory and effector memory RA T cells from CD4$^+$ or CD8$^+$ population. All results were analyzed by 3 independent donors. C) The representative FACS plots of CD45RA/CD62L expression from D12 samples.

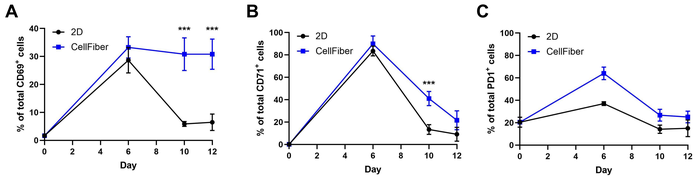

Figure 4 Fiber culture maintains significantly higher activation phenotype while performing low exhaustion phenotype after cell expansion compared to 2D culture
A & B & C) % of CD69$^+$ (early activation marker), CD71$^+$ (late activation marker) or PD1$^+$ (exhaustion marker) T cells from either fiber culture or 2D culture were analyzed by FACS. Cells were collected from anti-CD2/3/28 activator condition at indicated time points. All results were analyzed by 3 independent donors. *** means P <0.001 by 2-way ANOVA.

Summary

- CellFiber technology performs consistent T cell production by 750-fold cell expansion in 12 days
- CellFiber culture shows higher CD8$^+$ naïve and CD4$^+$ central memory populations compared to 2D condition
- Fiber-cultured T cells maintain higher activation phenotype and low exhaustion phenotype after cell expansion
- CellFiber platform performs comparable T cell production in large-scale process with bioreactor (data no shown)