Novel 3D Culture Strategy of Stem Cells Using Alginate Hydrogel Encapsulation Technology

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Introduction

Cell therapy manufacturing is increasingly constrained by the scalability limits of monolayer (2D) culture — particularly for allogeneic MSC products requiring batch sizes in the 10^9–10^{10} cell range, and iPSC-derived products requiring large undifferentiated cell banks prior to lineage commitment. Suspension-based 3D approaches (microcarriers, aggregates, encapsulation) address scale, but each introduces trade-offs in shear protection, aggregation control, or harvest complexity. While CellFiber®'s encapsulation platform is established for MSC bioprocessing, its applicability to pluripotent stem cells — a substantially more sensitive cell type — has not previously been demonstrated. This work presents validation of CellFiber® fiber culture for iPSC expansion, alongside updated MSC manufacturing economics.

Method

[MSC]
Human Mesenchymal Stem Cells were recovered from cryopreservation and pre-cultured for 7 days in T75 flasks. The collected MSCs were encapsulated in the tubular alginate hydrogel for 8-day fiber culture in a 10 L cellbag bioreactor (Xuri™ W25 cell expansion system, Cytiva).
[iPSC]
Cryopreserved induced Pluripotent Stem Cells were thawed and pre-cultured under adherent conditions for 13 days, then encapsulated and cultured in fiber format for 7 days in a 2 L Cellbag bioreactor (Xuri™ W25 cell expansion system, Cytiva).
After fiber culture, expanded cells are harvested by dissolving hydrogel shells with a chelating buffer, followed by washing with an automated cell harvesting system. Pluripotency and surface marker expression are assessed by flow cytometry and qRT-PCR. (Figure 2).

Results

[MSC]
The MSC culture yielded 1.10 × 10^9 cells (70.5-fold expansion) at 97.5% viability. Flow cytometry confirmed high expression of CD73 (99.98%) and CD105 (98.30%), with low expression (<1.1%) of the negative marker panel (CD11b, CD19, CD34, CD45, HLA-DR), consistent with established MSC surface phenotype. Modeled cost analysis projects up to 70% lower manufacturing cost versus 2D culture at the expected batch yield of 1.10 × 10^{10} BM-MSCs, driven primarily by reductions in labor (50%) and consumables (40%) relative to facility cost (Figure 3).
[iPSC]
The culture at 1L-scale yielded 1.11 × 10^{10} iPSCs. The overall expansion fold during the culture period was 55.5-fold, and cell viability at harvest reached 97.4%. Key pluripotency markers, including OCT3/4, NANOG, SSEA-4, and TRA-1-60, remain highly expressed after expansion. This indicates that the cells maintain an undifferentiated state. Trilineage differentiation was conducted, and qRT-PCR analysis confirmed the expression of lineage-specific markers for ectoderm (SOX2), mesoderm (Brachyury), and endoderm (FOXA2), all upregulated significantly relative to 2D culture. (Figure 4). Preliminary in-house data on cardiomyocyte differentiation further support CellFiber's potential to enhance directed differentiation (data not shown).

Conclusion

The CellFiber® platform integrates automated, closed-system processing from encapsulation through expansion and harvest. Preliminary trials encapsulating cells directly, without preculture, demonstrate successful growth (data not shown), suggesting the manufacturing workflow can be further streamlined. Combined with the yield, viability, and cost advantages shown here, CellFiber® is well-positioned for adaptation into GMP manufacturing environments.

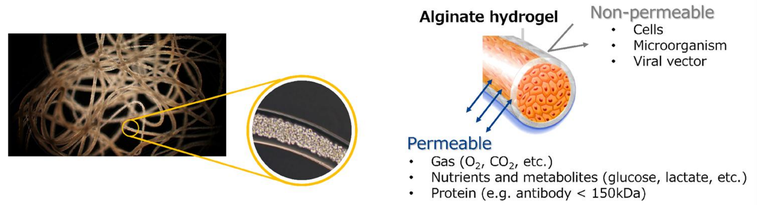

Figure 1. CellFiber® cell encapsulation technology

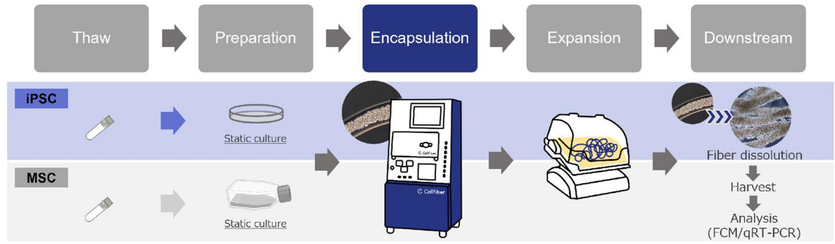

Figure 2. CellFiber integrated Stem cell manufacturing platform

A

	Cell numbers	Viability	Expansion fold
Encapsulation	1.56 x 10^7	96.8%	-
Harvest	1.10 x 10^9	97.5%	70.5

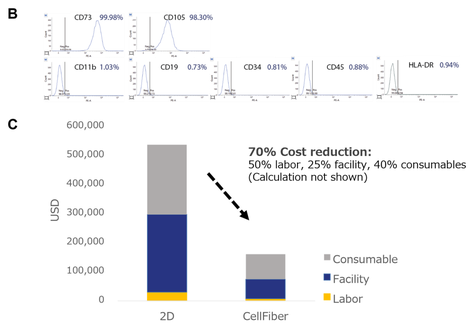

Figure 3. Yield, Quality of 5-litre culture of BM-MSC Fiber culture

A

	Cell numbers	Viability	Expansion fold
Encapsulation	2.00 x 10^8	98.8%	-
Harvest	1.11 x 10^{10}	98.4%	55.5

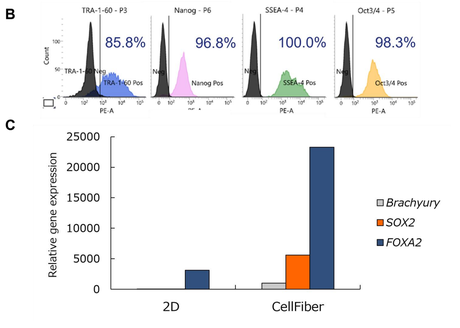

Figure 4. Yield and Pluripotency of 1-litre iPSC Fiber culture

CellFiber
Cell Culture Reimagined