Development of an Automated, Closed-System MSC Manufacturing Platform Using CellFiber Technology

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Background and Objectives

CellFiber technology is an encapsulation method that protects cells within a tubular structure composed of medical-grade alginate. A proprietary microfluidic device enables simultaneous fiber formation and encapsulation of the cell suspension (Fig. 1). Mesenchymal stem cells (MSCs) expansion is limited by available adherent surface area, and conventional multi-layer flask methods are labor-intensive and time-consuming. This study aimed to develop a large-culture process for manufacturing MSCs for cell therapy within a closed-system environment in a cleanroom using CellFiber technology. Large-scale cultivation using CellFiber technology allows for the establishment of a closed-system culture process, significantly reducing the risk of contamination.

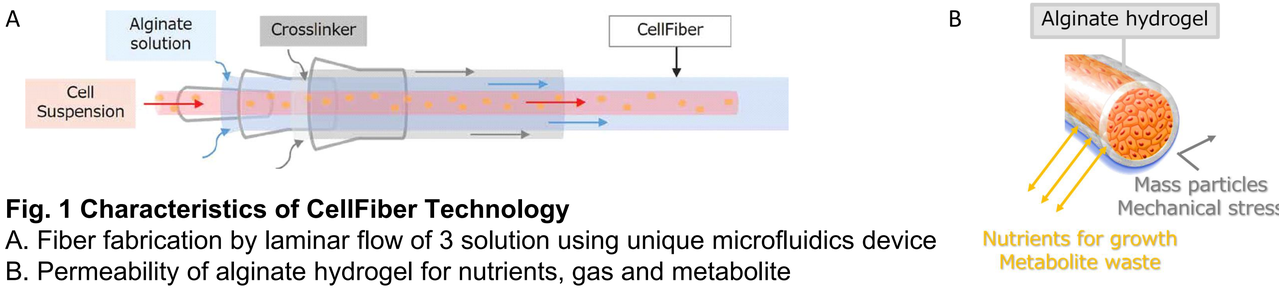


Fig. 1 Characteristics of CellFiber Technology
A. Fiber fabrication by laminar flow of 3 solution using unique microfluidics device
B. Permeability of alginate hydrogel for nutrients, gas and metabolite

Methodology

- BM-MSCs were pre-cultured in T75 flasks for 4 days (2D adherent culture)
- Cells were encapsulated into fibers and expanded in a 1L gas-permeable bag (GPB) for 7 days
- Cells were harvested and prepared for scale-up using a closed Sepax C-pro system
- Fibers for 10L culture were fabricated and cultured in a 20L CellBag using Xuri system for 8 days
- Medium perfusion initiated on Day 12 and increased from 2.0 to 4.0 L/day
- Adhesion Enhancer (AE) added from Day 14 to maintain fiber integrity
- Fibers were dissolved after culture, and cells were recovered using LOVO system (Fig.3)
- Cell count and MSC surface markers were analyzed post-culture

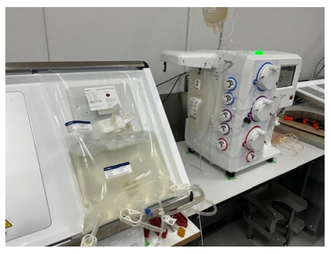


Fig. 3 Cell harvesting with LOVO

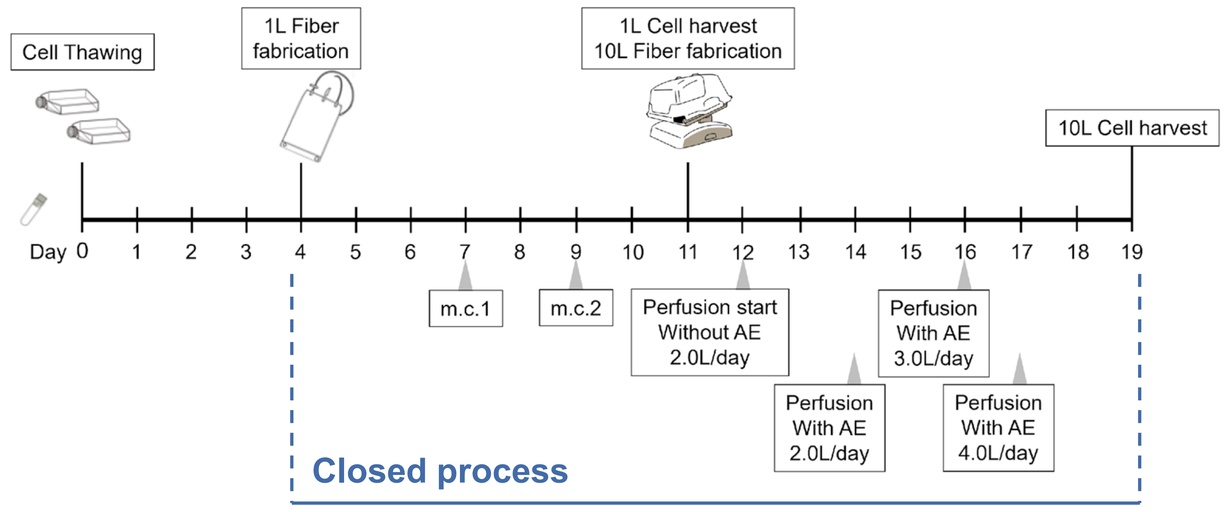
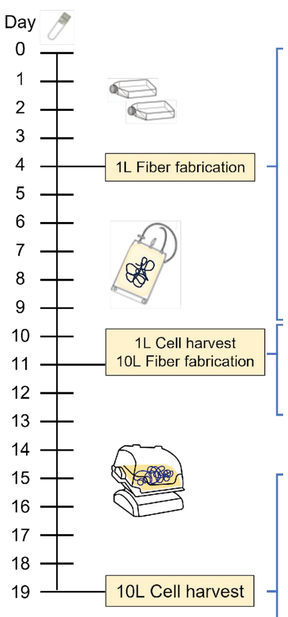


Fig. 2 Process overview of 10L-MSC culture with CellFiber technology

Results

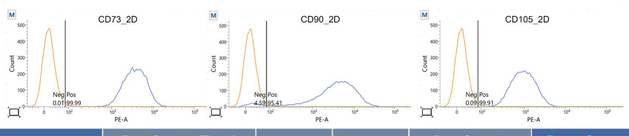


Day4	Seed Cell Number (cells)	Viability (%)	Harvested Total Live Cell Number (cells)	Viability (%)	Proliferation (-fold)	Doubling time (hrs.)
T75 flask x 2	1.02 × 10^6	95.4	6.60 × 10^6	97.9	6.5	35.6

a

Day4	CD11b	CD19	CD34	CD45	HLA-DR	CD73	CD90	CD105	HLA-ABC
T75 flask	1.01	0.64	0.97	1.02	2.34	99.99	95.41	99.91	99.89

i



A

Day11	Pre-Sepax Total Live Cell Number (cells)	Viability (%)	Volume (mL)	Pre-Sepax Doubling time (hrs.)	Post-Sepax Total Live Cell Number (cells)	Viability (%)	Volume (mL)	Harvest Efficiency (%)	Post-Sepax Proliferation (-fold)
GPB1000mL	1.55 × 10^8	94.0	950	32.2	9.86 × 10^7	96.2	100	63.6	23.7

B

Day19	Pre-LOVO Total Live Cell Number (cells)	Viability (%)	Volume (mL)	Pre-LOVO Doubling time (hrs.)	Post-LOVO Total Live Cell Number (cells)	Viability (%)	Volume (mL)	Harvest Efficiency (%)	Post-LOVO Proliferation (-fold)
CellBag20L	4.31 × 10^9	90.8	4500	28.3	1.11 × 10^9	93.9	200	25.8	28.5

b

Day19	CD11b	CD19	CD34	CD45	HLA-DR	CD73	CD90	CD105	HLA-ABC
CellBag20L	0.71	0.65	0.63	1.01	0.85	99.98	89.60	99.75	98.96

ii

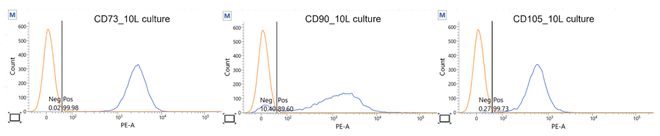


Fig. 4 Overall result of proliferation and quality of expanded BM-MSC.
Following the 7-day culture in the GPB 1000 mL, the fibers were dissolved, and cells were dissociated into single cells using TrypLE Select. Cells were harvested using the Sepax C-pro system (Cytiva); the cell count measured by an automated cell counter was 9.86x10^7 cells (pre-harvest count: 1.55x10^8 cells; recovery rate: 63.6%) (Table A in Fig. 4).

A portion of these harvested cells was passaged into fibers for a 10L-scale culture and maintained for 8 days. After the 10L culture, cells were dissociated into single cells (consistent with the 1000 mL GPB protocol) and harvested using the LOVO system (Fresenius Kabi). The final cell count was 1.11x10^9 cells (pre-harvest count: 4.31x10^9 cells; recovery rate: 25.8%) (Table B in Fig. 4). For both the 2D culture and each fiber culture stage (calculated based on pre-harvest cell counts), the doubling time was approximately 30 hours.

Analysis of cell surface marker confirmed that cells from both the 2D culture and the 10L fiber culture maintained characteristic MSC functionality (Tables a & b and Fig. i & ii in Figs. 4). (Due to the specific cell lot used, a certain population of CD90-negative cells was observed in the negative region. We have confirmed through fiber culture of MSCs from other sources that this phenomenon is not caused by the fiber culture process itself.)

In a separate run of this process, it was confirmed that cells harvested after 10L fiber culture retained the multipotency to differentiate into chondrocytes, osteoblasts, and adipocytes (Fig. 6).

Fig. 5 microscopic observation of BM-MSC in fiber. This photograph was taken on Day 11 of the MSCs cultured in 1000 mL of GPB prior to harvesting.

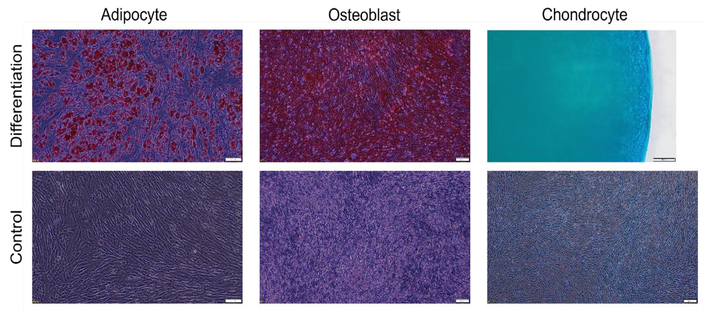


Fig. 6 Confirmation of BM-MSC Differentiation Potential
In a separate run of this process, it was confirmed that cells harvested after 10L fiber culture retained the multipotency to differentiate into chondrocytes, osteoblasts, and adipocytes

Table.1 Estimation of surface area of the Fiber
The adhesion surface area of the fiber fabricated inside the GPB1000mL is comparable to that of the 10-layer flask. The total adhesion surface area of the fabricated fibers inside the CellBag20L is equivalent to that of 10 10-layer flasks.

	Standard multi-layer flask		CellFiber Technology	
Culture scale	5 layers	10 layers	1L culture in GPB1000mL	10L culture in CellBag20L
Adhesive surface area (cm^2)	3180	6360	6656*	62410*

*calculated based on the fiber inner diameter and fiber internal volume

Discussion

- ✓ The relatively low recovery rate is likely due to alginate-derived viscosity, highlighting the need for parameter optimization, which is currently underway to improve performance.
- ✓ Cell expansion was comparable to literature using a 20L CellBag and rocking bioreactor [1].
- ✓ Dissolved oxygen (DO) decreased to ~74% by Day 19 without control.
- ✓ The impact of DO on cell growth and quality remains to be fully evaluated.

Reference
1. Hassan et al., 2020, “Large-Scale Expansion of Human Mesenchymal Stem Cells”, Stem Cells International, Volume 2020, Issue 1.

Conclusion

- ✓ Fully closed manufacturing from 1L to 10L, including all passage operations
- ✓ Reduced contamination risk with a flexible manufacturing approach
- ✓ Scalable and practical for large-scale MSC manufacturing
- ✓ Ongoing optimization and reproducibility validation for 10L process
- ✓ *~60% cost reduction potential vs. conventional multi-layer flask methods (including labor, facility, and consumables)

*in-house estimation