

Up to 151 Millionfold Cumulated Expansion of Encapsulated hiPS Cells in Bioreactor Over 28 Days: Comparison with 2D Culture and Standard Spheroid Culture

Authors

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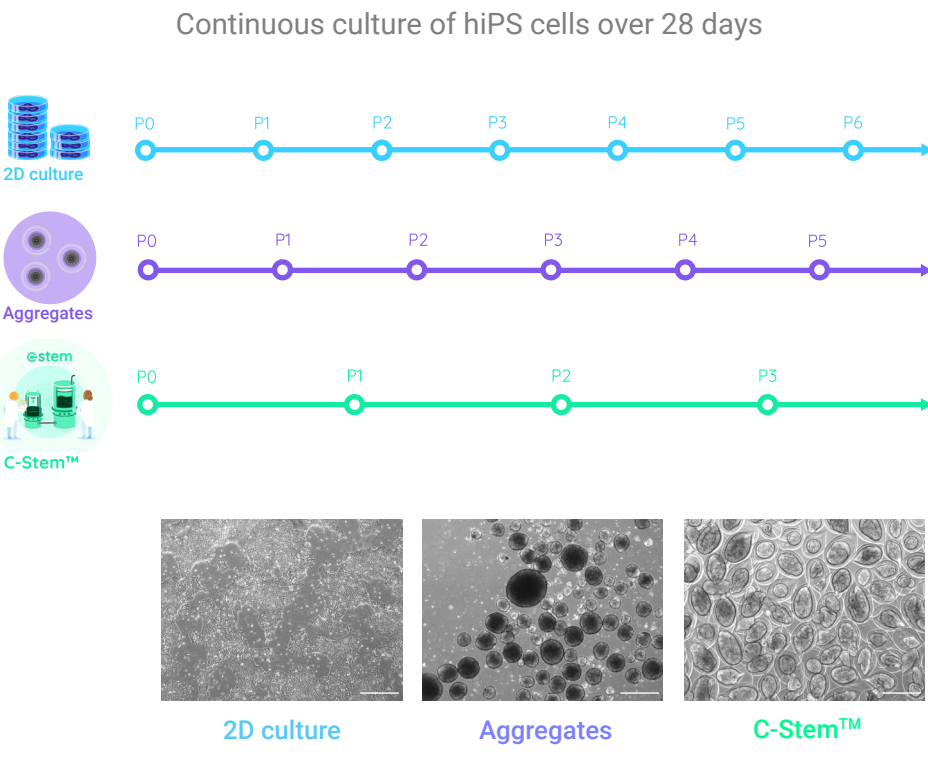
Introduction

By combining stem cell biology and microfluidics, TreeFrog Therapeutics has developed a high-throughput cell-encapsulation process (C-Stem™) that provides a biomimetic 3D culture environment, where cells self-organize in an epiblast structure similar to early embryonic development.

Here we compare the growth of iPS cells in 2D conventional culture vs aggregates vs. encapsulated cells (C-Stem™) grown in bioreactors during a period of 28 days.

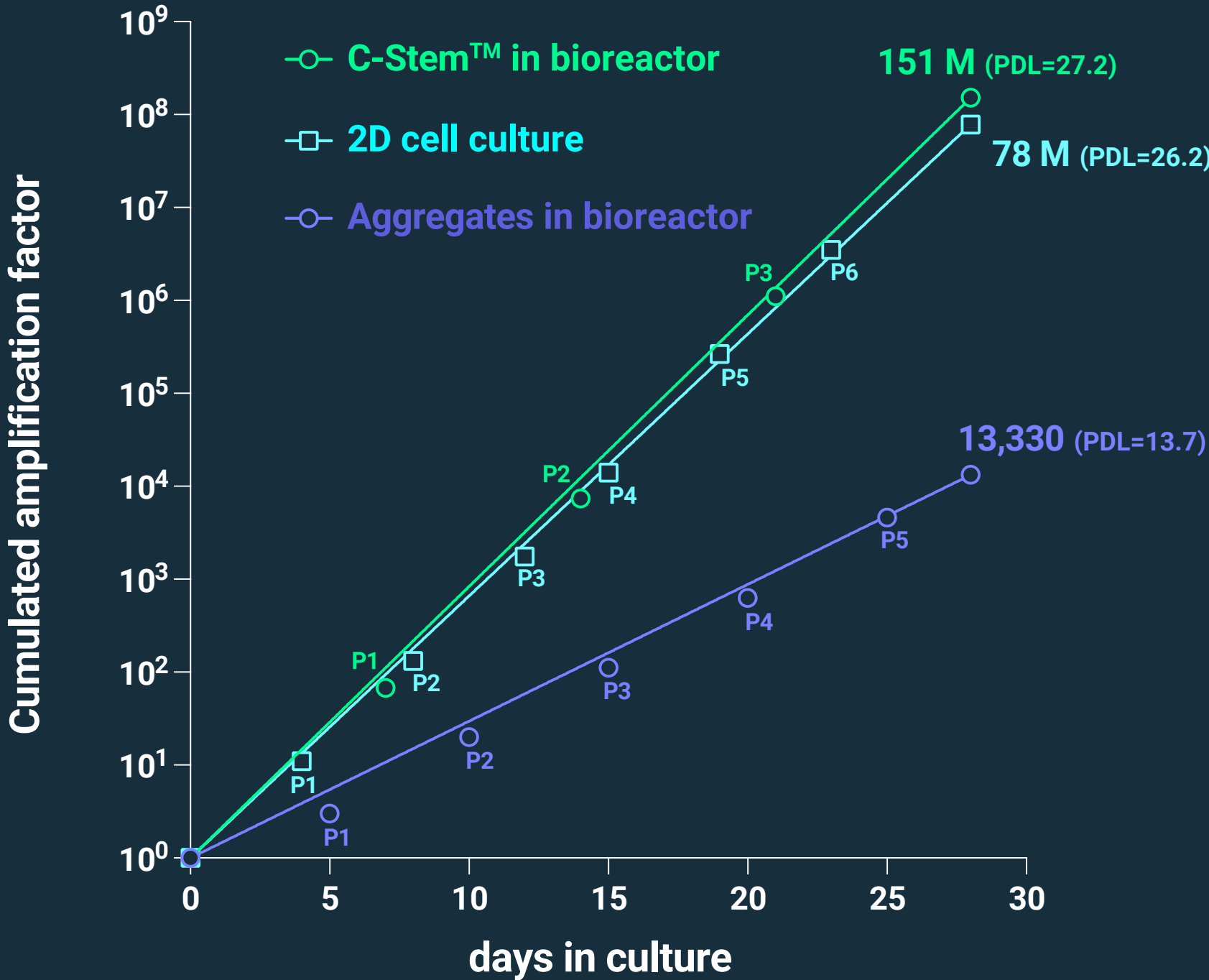
Methods

The iPS cell line IMAGINi005 previously characterized¹ at Imagine Institute iPS platform was used.



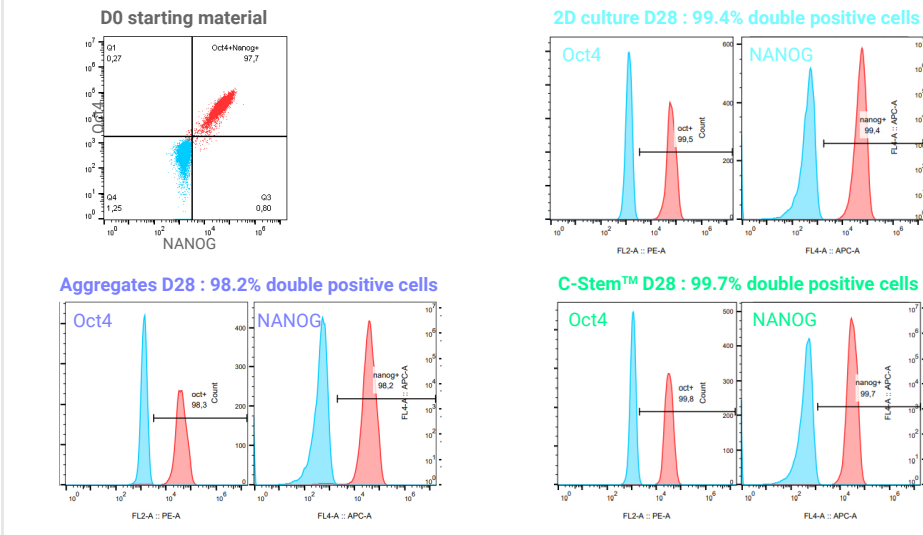
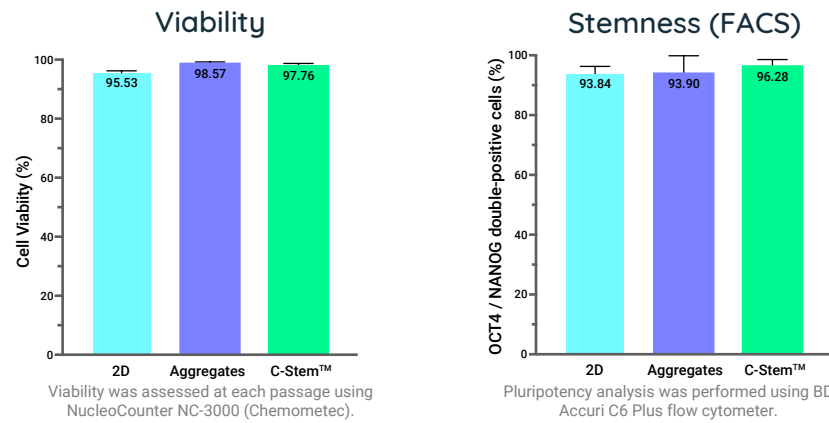
Seeding density	10,000-30,000 cells/cm²	175,000 cells/mL	NC
Culture vessel	T75, Matrigel coating	30mL Able Bioreactor	30mL Able Bioreactor
Passage	at 80% confluency, every 3 to 5 days: non-enzymatic clumps method with 24h ROCK-inhibitor treatment	every 5 days: enzymatic dissociation with 24h ROCK-inhibitor treatment	every 7 days: capsule dissolution followed by enzymatic dissociation, with 24h ROCK-inhibitor treatment

C-Stem™ provides best hiPSC amplification over 28 days while controlling passage numbers



The mean amplification per passage was 14.1 ± 4.9 sd, 5.3 ± 2.2 , and 116 ± 36 for 2D culture, aggregates, and C-Stem™ respectively.

hiPSC quality over 28 days



Conclusion

C-Stem™ is capable of excellent phenotype maintenance over serial encapsulation passaging and a long period of culture. Comparatively, we have shown a growth advantage with C-Stem™, while reducing the number of passages. Protection of 3D stem cell colonies inside shielding capsules enables similar results and full scalability in much larger bioreactors (single batch of 15B hiPS cells in 10L bioreactor recently announced). Finally, C-Stem™ combines good amplification AND scalability in bioreactor, thus resolving the bottleneck of stem cell manufacturing.

Perspectives

Preliminary genetic analyses suggest that C-Stem™ allows maintaining the genomic integrity of hiPSC throughout large-scale amplification, in contrast with conventional 2D cultures and aggregate suspensions. The QC-Stem consortium, bringing together experts in the quality control of hiPS cells for clinical use from the Havard Stem Cell Institute and the Dana Farber Institute (USA), the FBRI (Japan), and the Imagine Institute (France), will further investigate the genomic safety of hiPS cells amplified with the C-Stem™ technology in the coming months.

