iPSC Expansion in a Stirred-Tank Bioreactor: Optimization and Automation of Feeding Strategies to Achieve Economic Feasibility

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Introduction

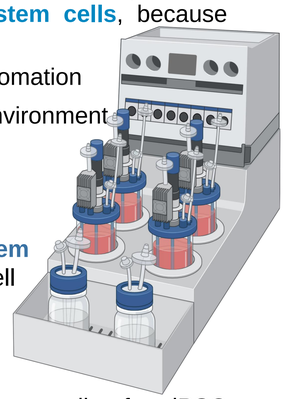
Large-scale expansion of human pluripotent stem cells is an evolving field with promising applications in cell therapy and regenerative medicine. Despite significant advances, economic viability of large-scale stem cell culture bioprocesses remains challenging, hampering translation. Cost of cell **culture media** is a major barrier.

The current work sought to answer the following question: **How does optimizing culture medium composition, perfusion culture, and online monitoring of glucose & lactate concentrations help to reduce culture media associated costs?** To answer, scientists at IST performed experiments using the DASbox® Mini Bioreactor System, culturing iPSCs as free-floating scaffold-free aggregates. Central to this work is HiDef® S8, a new chemically defined, animal-free suspension medium developed specifically for the efficient expansion and maintenance of human pluripotent stem cells. Daily samples were drawn from the bioreactors for cell counting, viability measurements, and immuno-cytochemistry was performed for flow cytometry.

Background

Stirred-tank bioreactors are ideal culture platforms for the large-scale culture of **stem cells**, because they:

- Integrate monitoring and automation
- Better mimic a 3D cellular environment
- Facilitate high cell densities
- Are highly scalable



Human induced pluripotent stem cells (iPSCs) are a promising cell type for advancing clinical applications.

This work tested four culture media for iPSC expansion: **mTeSR1**, **E8** and two formulations from ***Defined Bioscience***: **HiDef-B8** and **HiDef-S8**.

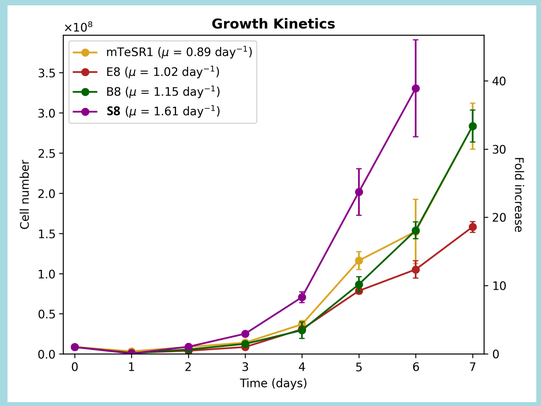
Materials and Methods

Experiments were performed using Eppendorf's DASbox® Mini Bioreactor System, culturing iPSCs as freefloating scaffold-free 3D aggregates:

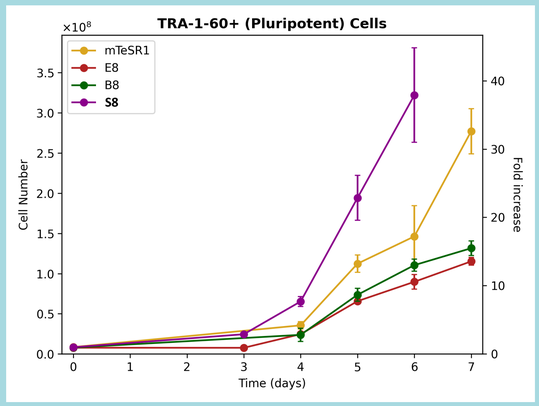
- Inoculation density of 100,000 iPSCs / mL
- Working volume of 80 mL
- Media exchange via continuous perfusion
- Monitoring of pH, DO, Temp, & glucose & lactate

Cell counts, viability staining for Calcein AM, and immunocytochemistry for FC performed daily.

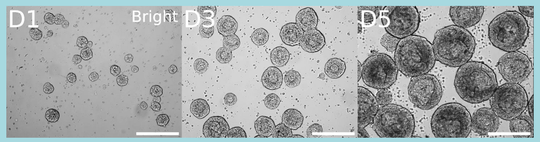
Results



mTeSR1 and **B8** led to fold expansions of 33× in 7 days, whereas **E8** led to a fold expansion of 18× in 7 days and **HiDef-S8** led to a fold expansion of 39× in 6 days. **mTeSR1** and **HiDef-S8** presented improved cell survival (38% and 37%, respectively). **HiDef-S8**'s growth rate was significantly faster than when using other culture media.



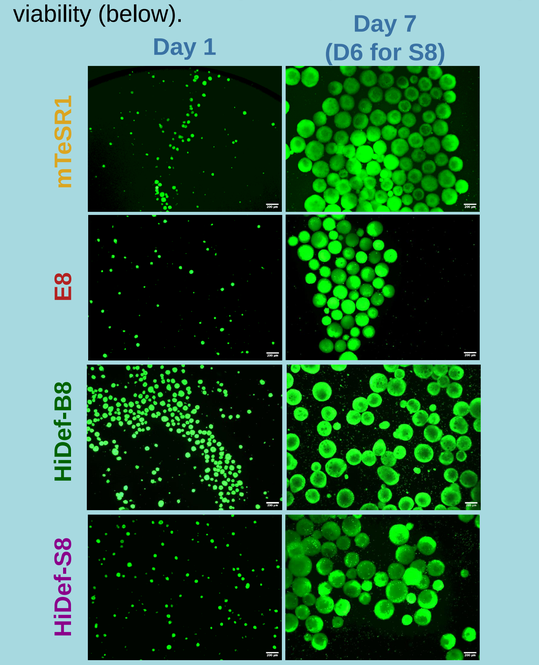
For pluripotency maintenance, only **mTeSR1** and **HiDef-S8** preserved high expression of key pluripotency markers (>95%). **E8** and **B8** both led to a significant decrease in TRA-1-60 expression at the end of culture.

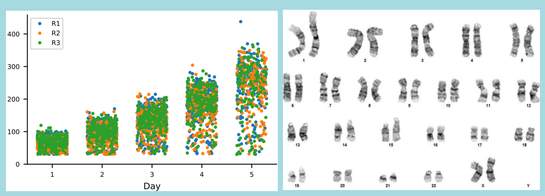


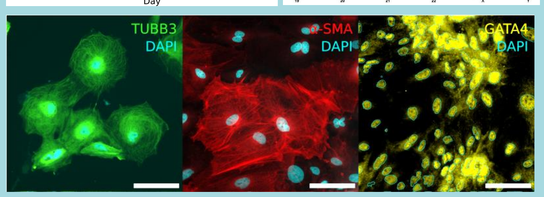
HiDef-S8 expanded aggregates present a hollow morphology (above). All aggregates stain for Calcein AM throughout expansion, indicating viability (below).

FC Marker	mTeSR1	E8	B8	HiDef-S8
OCT4	97.0	96.5	86.2	95.2
SOX2	98.0	99.0	96.0	99.3
TRA160	97.8	72.9	46.4	97.4
SSEA-4	98.2	97.9	97.8	98.4

mTeSR1 and **HiDef-S8** led to greater iPSC (TRA-1-60+) yields than **E8** and **B8**. Despite the similar performance of **mTeSR1** and **HiDef-S8** in this metric, **HiDef-S8**'s significantly lower cost and faster growth rate led to >34% cost reduction.







iPSC expanded in **HiDef-S8** over multiple iterations, and multiple passages maintain aggregate diameter below 400 µm, present normal karyotype, and readily differentiated into all three germ layers in adherent culture post-3D expansion.

Conclusions

For all media conditions, aggregates presented a hollow morphology and stained positive for Calcein AM viability marker throughout 6-7 days. **HiDef-S8** led to a fold expansion of 39× in 6 days. HiDef®-B8® and TeSR™ both led to fold expansions of 33× in 7 days, and E8 led to a fold expansion of only 18× in 7 days. TeSR and S8 presented improved cell survival.

S8 supported faster expansion and consumed volumes less medium. **HiDef-S8** and TeSR led to much greater TRA-1-60+ yields over 6 and 7 days, respectively, than either E8 or B8. Despite the similar performance of TeSR and S8 in this metric, S8's **lower cost per volume** and **faster stable growth rate** resulted in **substantially lower medium cost**. In terms of pluripotency maintenance, only **HiDef-S8** and TeSR preserved high expression of key pluripotency markers (>95%) after 6 days and 7 days, respectively. Notably, for extracellular vesicle production it is important that the culture medium contain minimal particles. Nanoparticle analysis showed that **TeSR contained substantially higher concentrations of particles** across multiple lots compared to **HiDef-S8**, **HiDef-B8**. HiDef-S8, a new suspension medium specifically optimized to support high-density PSC expansion, led to a 34% reduction in culture media associated costs comparatively to TeSR. Perfusion and glucose/lactate monitoring also decreases costs, by facilitating an optimal medium exchange rate.

Future Work

Future work will focus on manufacturing cGMP-compliant HiDef-S8 to support clinical applications. Defined Bioscience aims to expand their 3D culture platform to enable differentiation of human PSCs in suspension to further enhance scalability and efficiency for large-scale cell production.

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