Transforming Growth Factor Beta-3 in Suspension Culture Media for Induced Pluripotent Stem Cells

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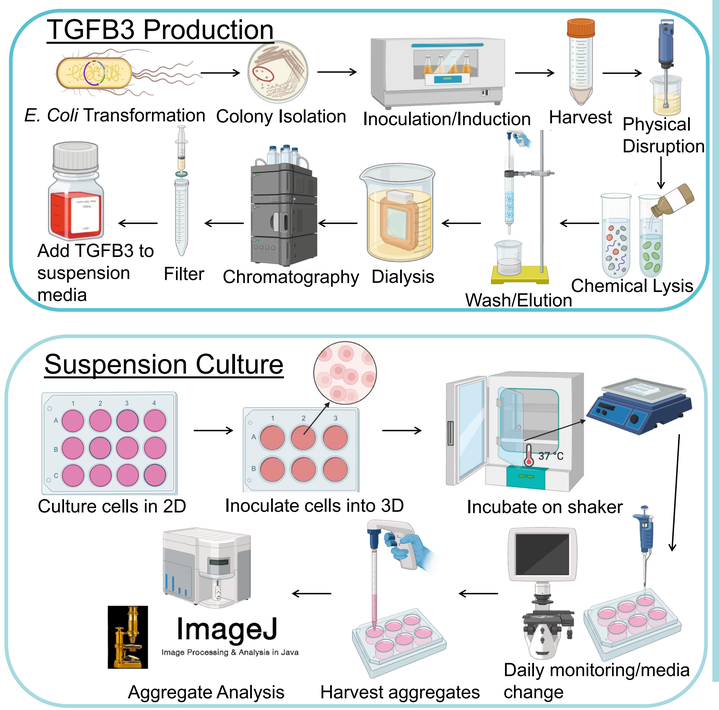
Introduction

Growth factors used in stem cell culture media account for approximately 80% of cost, making it one of the highest cost drivers of cell therapy and in-vitro biomedical research. Transforming growth factor beta (TGFβ) is a family of proteins that promote cell growth, embryogenesis, and differentiation and is essential in pluripotent stem cell medium formulations to support survival and proliferation. TGFβ-1 is most used in medium formulations, however, its signal promotes spontaneous differentiation and culture heterogeneity over extended expansion. TGFβ-3 activates the same SMAD2/3 pathway but supports pluripotency maintenance at a twenty-fold lower working concentration, resulting in long-term culture robustness and reduced costs. We expressed recombinant TGFβ-3 in *E. coli* and purified it using chromatography. Then, we compared effects in suspension culture by analyzing aggregate morphology, size, expansion, and pluripotency.

Methods



Results

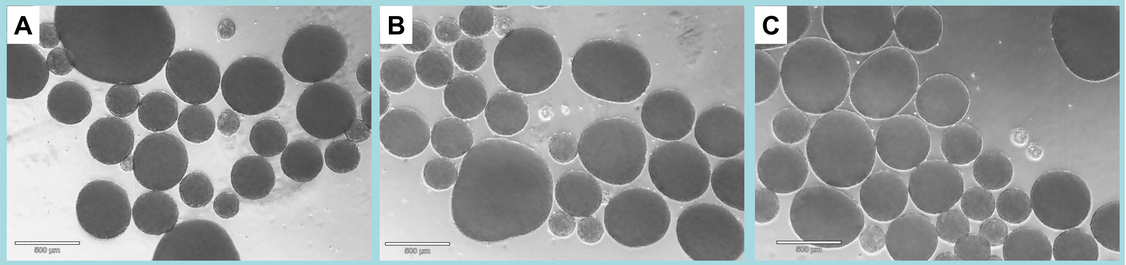


Figure 1: Images of iPSCs on day 4 of suspension culture (4X magnification). Similar morphology of aggregates (round, defined borders, no hollowness) in S8 (A), S8 with lower TGFβ-3 (B), and S8 with in-house TGFβ-3 (C) was seen.

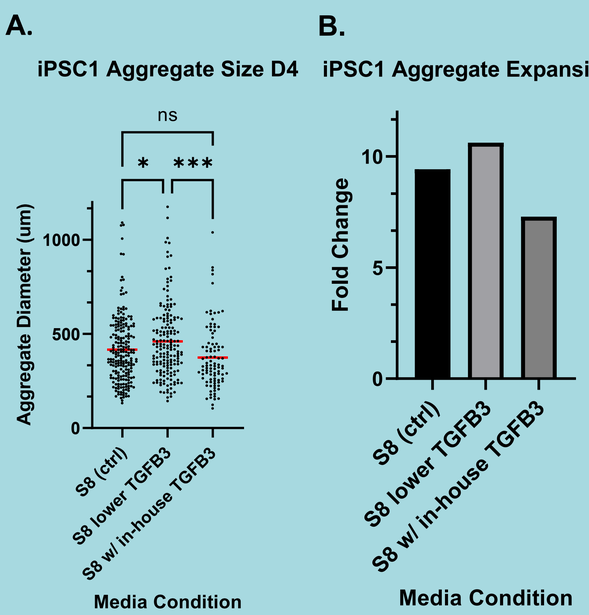


Figure 2: Aggregate Size and Expansion Analysis. iPSCs were cultured for four days in Defined Bioscience's suspension culture medium (HiDef® S8®). The width of individual aggregates were measured using ImageJ software on Day 4 (A). The fold change was also assessed (B). Decreasing TGFβ-3 yielded significantly larger aggregates ($p<0.05$) and highest expansion.

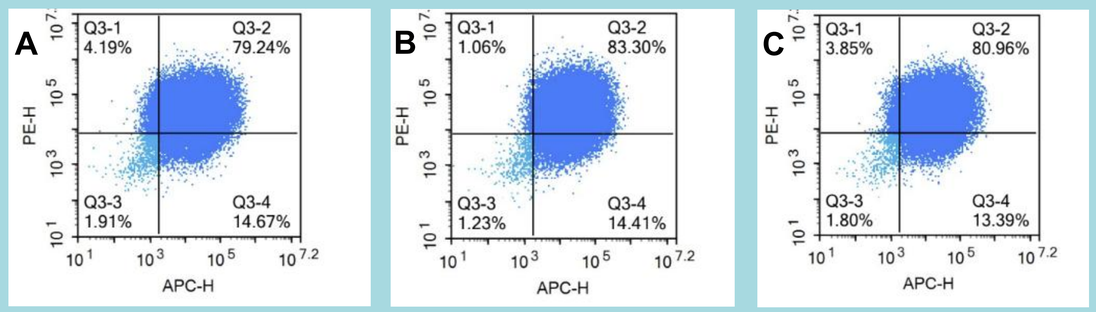


Figure 3: Pluripotency Analysis. Day 4 iPSC aggregates were stained with SSEA-4 conjugated with APC and TRA-1-60 conjugated with PE antibodies. Samples were analyzed with the Novocyte flow cytometer. Results indicate similar levels of SSEA-4 and TRA-1-60 expression across S8 (A), S8 with lower TGFβ-3 (B), and S8 with in-house TGFβ-3 (C).

Conclusions and Future Directions

Conclusions:

- TGFβ-3 was produced via recombinant expression in *E. Coli*
- Aggregates across all conditions had similar morphology
- Lowering TGFβ-3 maintains similar levels of pluripotency expression while having the highest fold change
- Using in-house TGFβ-3 did not have an impact on aggregate size, while maintaining pluripotency

Future Directions:

- Assess iPSC aggregate differentiation potential
- Optimization of suspension culture by modulating shear stress
- Optimizing production and purification of in-house TGFβ-3
- Serial expansion of iPSC aggregates
- Frozen down cell aggregates for easy thaw directly into suspension

References

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Acknowledgments

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