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Scalable Platform for Large-Scale Production of Human iPSCs Using an Automated Stirred Tank Bioreactor System for Bioprinting Applications

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This study was led by researchers at Stanford Bioengineering in collaboration with scientists at Sartorius.

Introduction and Key Highlights

- **hiPSCs for tissue engineering:** Human induced pluripotent stem cells (hiPSCs) play a pivotal role in tissue engineering, enabling scientists to create functional tissue for transplantation and disease modeling. Organ-scale tissue engineering requires generating large cell volumes – particularly wholly cellular bioinks for bioprinting, which require billions of human cells.
- **Challenges:** Conventional 2D culture methods lack scalability due to cost, space, and handling constraints.
- **Approach:** To overcome these challenges, our study developed an efficient and scalable platform using the following methodology:
  - Process development and optimization: Using MODDE® software, we implemented a data-driven design-of-experiments (DOE) approach to optimize hiPSC aggregate culture using NutriStem® hPSC XF medium in our automated stirred tank bioreactor system.
  - Process scale-up: We seamlessly scaled the optimized parameters from 250 mL to 1 L using Ambr® 250 Modular and Biostat® Univessel® Glass 2 L stirred tank bioreactors.

Experimental Overview: Bioprinting Process Workflow

- Figure 1 outlines the streamlined bioprinting process workflow offering both scalability and differentiation capabilities.
- The hiPSC aggregates (hAs) are expanded using automated stirred tank systems. Subsequently, the aggregates are harvested and centrifuged to create a concentrated bioink. The hAs bioink is then bioprinted in a collagen-Matrigel® matrix to construct the intended tissue or organ shape that can be further differentiated into cortical or vascular tissues.

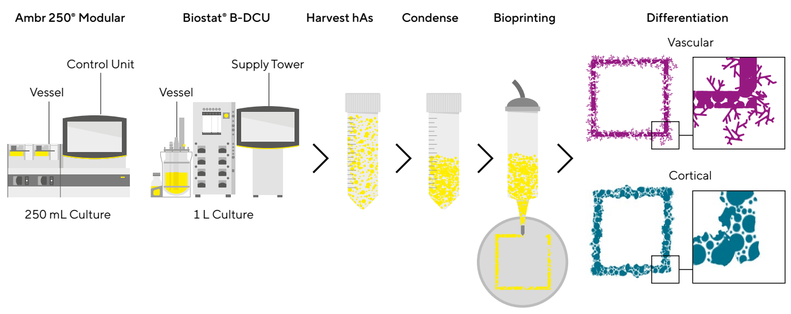

Figure 1: *hiPSC Aggregate Production Workflow for Bioprinting and Differentiation*

Method: Process Optimization

- A DOE approach using MODDE® software was employed to optimize bioreactor culture parameters as outlined in table 1.
- The process parameters tested included impeller initial speed, final speed, and seeding density in Ambr® 250 Modular stirred tank bioreactor system using NutriStem® hPSC XF medium supplemented with 40 ng/mL FGF.
- Highlighting S_{low}, S_{mid}, and S_{high} from the 15 tested conditions to illustrate the impact of impeller spin speed on hAs.

Experiment	Initial Speed [RPM]	Final Speed [RPM]	Seed Density [×10⁴ cells/mL]
S_a	150	250	190
S_b	200	250	187
S_b Repeat	200	250	166
S_{low}	100	250	117.5
S_c	150	250	191.5
S_d	200	300	89.76
S_e	200	300	155
S_f	200	400	153.6
S_f Repeat	200	400	110.04
S_g	200	400	217.5
S_h	300	400	119.5
S_{high}	200	500	204.12
S_{mid}	200	200	214.72
S_i	200	275	205.92
S_j	300	300	187

Note. Experiments designated S_a, S_b S_{low}, S_c have either low initial or final stirring speeds (100–250 RPM, initial impeller speed at 100 RPM then increased to 250 RPM over Days 1–5) or seeding densities. Experiments designated S_d, S_e, S_i, S_{mid} have either medium initial (constant speed at 200 RPM) or final stirring speeds or seeding densities. Experiments designated S_g, S_h, S_{high}, S_f, S_j have either high initial or final stirring speeds (200–500 RPM, initial impeller speed at 200 RPM on Day 0–1, then increased to 500 RPM over Days 1–5) or seeding densities.

Table 1: *Factor Settings for a Range of Parameters to Be Tested in a DOE*

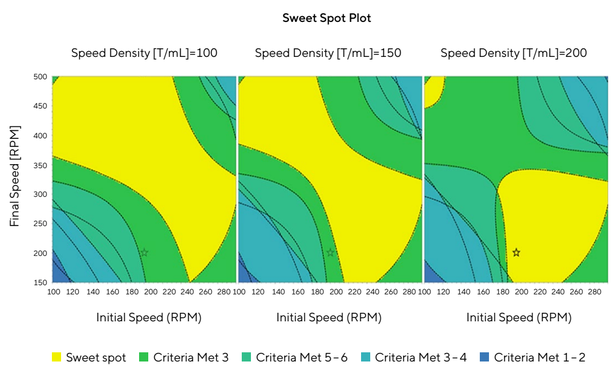

Figure 2: *Sweet Spot Plot for Predicting Optimal Culture Conditions Generated Using MODDE® DOE Software. Yellow Regions Indicate Where the Required Responses Are Met by the Parameter Settings*

Results

1. Process optimization: 1 billion hiPSC hAs by day 5

- **Efficient MODDE® software-driven optimization:** The S_{mid} condition resulted in a remarkable 23-fold cell expansion, yielding the highest cell density of 1 billion hiPSC hAs over five days at the 250 mL scale, with 96% viability and high pluripotency marker expression (>94% NANOG+, SSEA-4+, TRA-1-60+).
- **Optimal hA size and circularity:** The S_{mid} condition resulted in desired diameters for differentiation (250-300 µm) and maintenance (301-450 µm) with good circularity scores (~0.9).

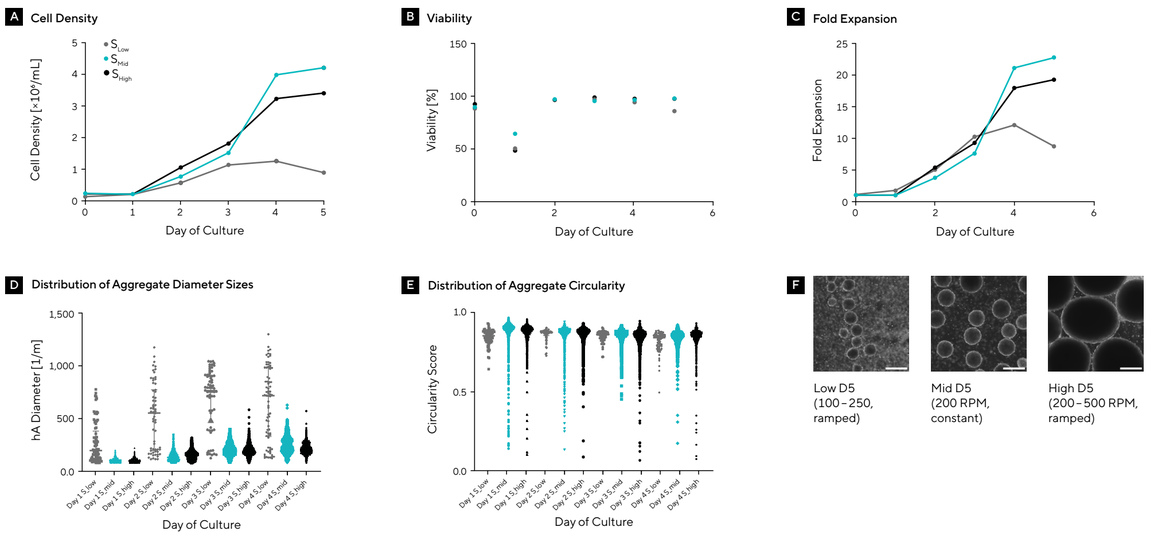

Figure 3: *Optimization of Impeller Speed and Seeding Density Parameters to Produce hiPSC Aggregates With Desired Characteristics for 3D Bioprinting and Differentiation*

2. Consistent growth and pluripotency in hAs across passages

- **Robust serial passaging of hAs:** Serial passaging of SCVI-15 and WTC-11 hiPSC cell lines across multiple passages showed consistent growth rates, morphologies, and uniform expression of pluripotency markers (OCT4, NANOG, and TRA-1-60) across hAs.

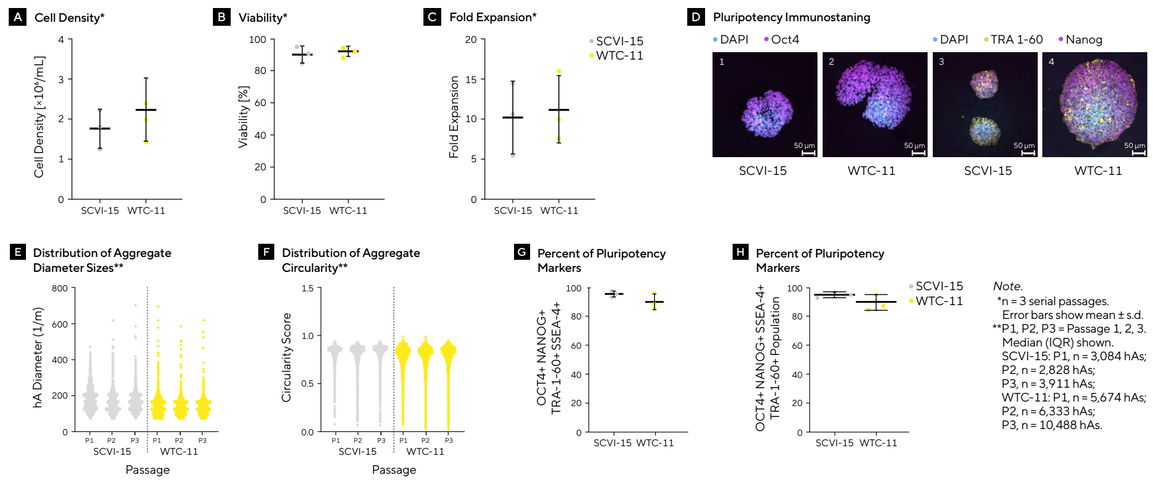

Figure 4: *hiPSC Aggregates Maintain Consistent Growth Rates and Morphologies When Cultured for Multiple Passages in Bioreactors*

3. Successful 1 L scale-up: ~4 billion hiPSC hAs by day 4

- **Consistent scalability:** Scaling up the culture to the Univessel® Glass 2 L bioreactor yielded consistent outcomes, with similar high cell density, viability, and aggregate formation for both SCVI-15 and WTC-11 hiPSC cell lines.
- **Successful process transfer:** The optimized process from Ambr® 250 Modular with NutriStem® hPSC XF medium transferred seamlessly to Univessel® Glass 2 L bioreactor, underscoring the reproducibility of the optimized process.

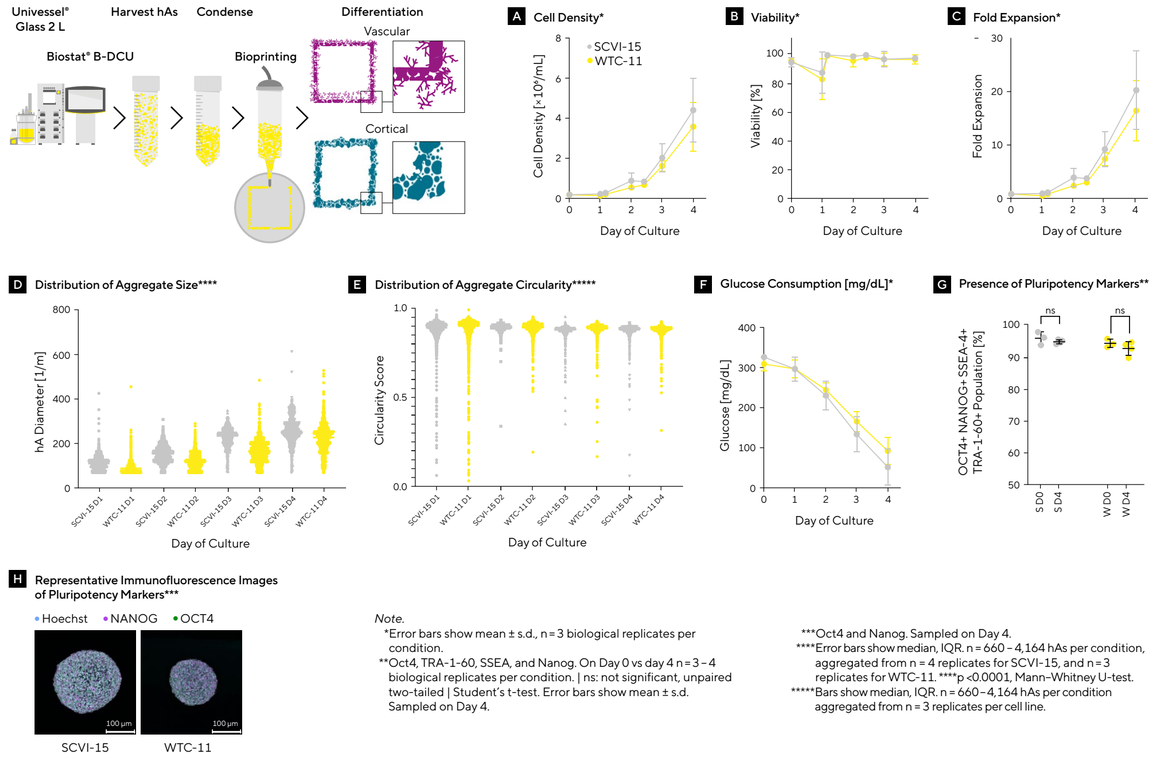

Figure 5: *hA Culture Can Be Robustly Scaled Up With Consistent Growth Rates, Morphologies, and High Rates of Pluripotency*

4. Effective trilineage differentiation potential of hiPSC hAs

- **Multi-germ layer organoid differentiation:** The successful differentiation of hAs into organoids representing ectoderm, mesoderm, and endoderm layers underscores their potential for enabling bioprinting across diverse tissue types.

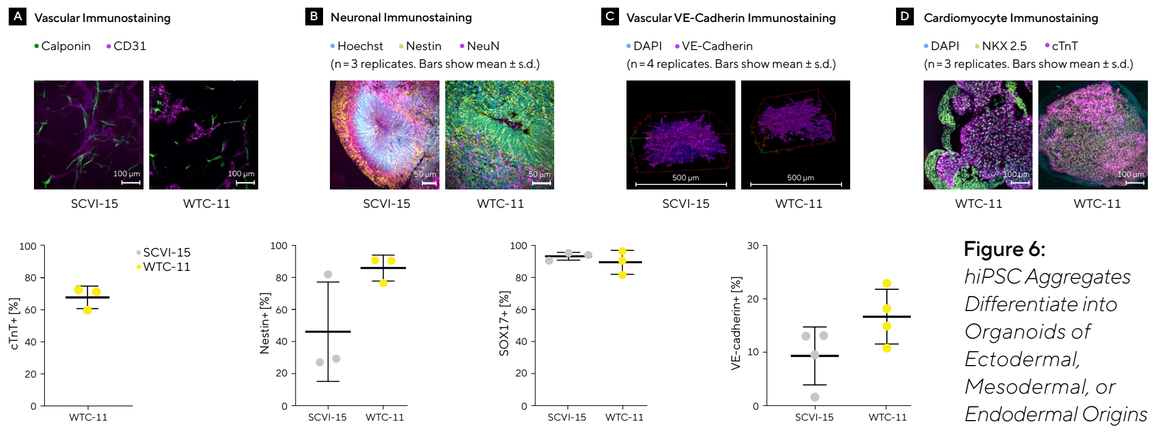

Figure 6: *hiPSC Aggregates Differentiate into Organoids of Ectodermal, Mesodermal, or Endodermal Origins*

Conclusion

- **Successful hiPSC-to-bioprinting platform:** Our study demonstrated a streamlined hiPSC-to-bioprinting pipeline, yielding scalable quantities of densely-cellular bioinks.
- **Optimized scalable process via MODDE® software:** Employing DOE using MODDE® software, we achieved 1 billion hAs in 5-day cultures using Ambr® 250 modular system, and ~4 billion hAs in 4-day cultures with Univessel® Glass 2 L bioreactor using NutriStem® PSC XF media.
- **Versatile organoid generation with therapeutic potential:** Cultured hAs formed organoids expressing markers from all germ layers with directed differentiation, implying potential for therapeutic-scale organ tissue production.