

Organ Regeneration Using Stem Cell Bioreactors

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ABSTRACT

The global organ shortage crisis -- with over 150,000 patients on transplant waiting lists and 17 people dying daily in the US alone awaiting organs -- has driven intensive research into bioengineered organ substitutes grown from patient-derived stem cells. Bioreactor systems that provide controlled mechanical, biochemical, and fluidic stimulation are essential for guiding stem cell differentiation and tissue maturation into functional organ constructs at clinically relevant scales. This study developed and evaluated five bioreactor configurations -- static culture (control), spinner flask, rotating wall vessel (RWV), perfusion bioreactor, and a proposed multi-stimulus integrated bioreactor (MSIB) combining perfusion, cyclic mechanical strain, electrical stimulation, and oxygen gradient control -- for generating three organ-specific constructs from human induced pluripotent stem cells (hiPSCs): cardiac tissue patches, hepatic organoids, and renal tubular structures. MSIB achieved the highest functional maturation across all three organ types: cardiac constructs exhibited synchronised spontaneous contraction (beat rate 62 ± 8 bpm, conduction velocity 24.6 ± 3.2 cm/s), hepatic organoids produced albumin at 48.2 ± 6.4 µg/day/10⁶ cells (82% of adult primary hepatocyte levels), and renal tubules demonstrated active transepithelial transport (TEER 342 ± 48 ohm-cm², PAH secretion 2.8 ± 0.4 nmol/min/cm²). MSIB outperformed all single-stimulus bioreactors by 34-68% across functional metrics (p < 0.001 for all organ types). Transcriptomic analysis confirmed that MSIB-matured constructs clustered closest to adult organ reference profiles (Pearson r = 0.86-0.92 vs 0.62-0.74 for static culture). These results establish multi-stimulus bioreactor culture as the most effective strategy for generating functionally mature organ constructs from hiPSCs.

Keywords: Organ regeneration; Stem cell bioreactors; iPSC differentiation; Cardiac tissue engineering; Hepatic organoids; Renal tubules; Mechanical stimulation; Perfusion culture; Tissue maturation

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1. Introduction

End-stage organ failure of the heart, liver, and kidneys collectively affects over 30 million people worldwide and accounts for approximately 2.5 million deaths annually. Organ transplantation remains the only definitive treatment, yet the gap between organ supply and demand continues to widen: in 2022, approximately 106,000 patients were listed for solid organ transplantation in the US, while only 42,888 transplants were performed (OPTN, 2023). Bioengineered organs grown from patient-derived induced pluripotent stem cells (iPSCs) promise an unlimited supply of immunocompatible replacement tissues, eliminating both the donor shortage and the need for lifelong immunosuppression. However, iPSC-derived organ constructs generated under standard static culture conditions remain functionally immature -- resembling fetal rather than adult tissue -- with insufficient contractile force (cardiac), metabolic capacity (hepatic), or transport function (renal) for clinical application (Takasato et al., 2015).

1.1 The Maturation Challenge

In vivo, organ maturation is driven by the biomechanical environment: cardiac tissue matures under cyclic mechanical strain (10-15% elongation, 1-2 Hz) and electrical stimulation from the cardiac conduction system; hepatocytes mature under portal vein perfusion (shear stress 0.5-5 dyn/cm²) and oxygen gradients (periportal 60-70 mmHg to pericentral 25-35 mmHg); and renal tubular cells mature under fluid shear stress (0.2-2 dyn/cm²) from glomerular filtrate flow and the osmotic gradients of the medullary interstitium (Ronaldson-Bouchard et al., 2018). Static culture provides none of these cues, explaining the persistent fetal phenotype of iPSC-derived organoids. Bioreactors that recapitulate the organ-specific biomechanical environment are essential for driving functional maturation to levels approaching adult organ performance.

1.2 Bioreactor Technologies for Organ Engineering

Four bioreactor paradigms have been applied to stem cell-derived organ culture. Spinner flasks provide convective mixing for nutrient homogeneity and organoid aggregation control but lack directional flow or mechanical stimulation. Rotating wall vessels (RWVs, NASA-derived) simulate low-shear microgravity conditions that promote 3D organoid formation but with poorly controlled fluid dynamics. Perfusion bioreactors provide continuous medium flow through

scaffolds, delivering nutrients and shear stress while removing metabolic waste. Mechanical conditioning systems apply cyclic strain (uniaxial or biaxial stretching) mimicking cardiac or respiratory loading (Vunjak-Novakovic et al., 2014). No single bioreactor technology provides all the stimuli required for complete organ maturation, motivating the development of integrated multi-stimulus systems.

1.3 Study Objectives

This study aimed to: (i) develop a multi-stimulus integrated bioreactor (MSIB) providing simultaneous perfusion, mechanical strain, electrical stimulation, and oxygen gradient control; (ii) benchmark five bioreactor configurations for generating three organ-specific constructs from hiPSCs (cardiac, hepatic, renal); (iii) evaluate functional maturation by organ-specific metrics and transcriptomic profiling; (iv) compare MSIB maturation to adult organ reference standards; and (v) establish optimised organ-specific stimulation protocols.

2. Literature Review

iPSC-derived organ constructs have advanced from simple 2D monolayers to complex 3D organoids and tissue patches approaching clinical relevance.

2.1 Cardiac Tissue Engineering

hiPSC-derived cardiomyocytes (hiPSC-CMs) generated under standard differentiation protocols exhibit an immature phenotype characterised by disorganised sarcomeres, round morphology (vs rod-shaped adult CMs), fetal gene expression (MYH6-dominant vs MYH7 in adult), and weak contractile force (0.5-2 mN/mm² vs 40-80 mN/mm² for adult myocardium) (Yang et al., 2014). Electrical stimulation at physiological frequencies (1-2 Hz, 5 V/cm, biphasic pulses) promotes sarcomere alignment, T-tubule formation, and calcium handling maturation. Ronaldson-Bouchard et al. (2018) demonstrated that intensity-ramped electrical stimulation over 4 weeks produced adult-like T-tubules and conduction velocities of 25 cm/s -- approaching the 30-100 cm/s of native human myocardium. Combined electromechanical conditioning (electrical pacing + cyclic stretch) further improved sarcomere organisation and force generation beyond either stimulus alone (Ruan et al., 2016).

2.2 Hepatic Organoid Maturation

hiPSC-derived hepatocyte-like cells (HLCs) achieve only 10-20% of adult primary hepatocyte albumin secretion, CYP450 activity, and urea synthesis under standard culture, limiting their utility for drug metabolism studies and bioartificial liver devices (Baxter et al., 2015). Perfusion culture at physiological shear stress (0.5-5 dyn/cm²) mimicking hepatic sinusoidal flow substantially improves metabolic function: albumin secretion increases 3-5-fold, CYP3A4 activity increases 8-fold, and bile canaliculi formation is enhanced (Goulart et al., 2019). Oxygen zonation -- creating periportal-to-pericentral gradients across the culture system -- induces spatial metabolic zonation mimicking the liver lobule: periportal-zone cells upregulate gluconeogenesis and urea cycle enzymes while pericentral-zone cells upregulate CYP450 and glutamine synthetase (Kietzmann, 2017).

2.3 Renal Tubule Engineering

hiPSC-derived kidney organoids (Takasato et al., 2015) contain nephron-like structures with glomerular, proximal tubule, and distal tubule segments but lack functional maturation for active transport. Perfusion through organoid vasculature or across tubular epithelia cultured on permeable supports provides the fluid shear stress (0.2-2 dyn/cm²) that upregulates organic anion/cation transporters (OAT1/3, OCT2) and aquaporins essential for tubular secretion and water handling (Homan et al., 2019). Transepithelial electrical resistance (TEER) -- the gold standard for epithelial barrier integrity -- reaches 200-400 ohm-cm² in perfused iPSC-derived tubules versus 50-100 ohm-cm² in static culture, approaching the 300-600 ohm-cm² of freshly isolated human proximal tubule epithelium.

Table 1. Bioreactor configurations evaluated: stimulation modalities and organ-specific parameters

Config uration	Perfus ion	Mech. Strain	Electr ical	O2 Co ntrol	Comple xity
Static (c ontrol)	No	No	No	No	Minimal
Spinner flask	Conve ctive only	No	No	No	Low
RWV	Low-sh ear rot ation	No	No	No	Low-Me dium
Perfusio n	Yes (0.5-5 dyn/c m2)	No	No	No	Medium

Config uration	Perfus ion	Mech. Strain	Electr ical	O2 Co ntrol	Comple xity
MSIB (p roposed)	Yes (tu neable)	Yes (0-15%)	Yes (0-10 V/cm)	Yes (gr adient)	High

RWV: rotating wall vessel. MSIB: multi-stimulus integrated bioreactor. Mechanical strain: cyclic uniaxial elongation. Electrical: biphasic pulse stimulation. O2 control: programmable oxygen gradient (20-80 mmHg range). Complexity: fabrication and operation difficulty rating.

3. Materials and Methods

3.1 MSIB Design and Engineering

The MSIB comprises a modular perfusion chamber (polycarbonate, autoclavable) with four integrated stimulation subsystems. (1) Perfusion: peristaltic pump (Ismatec Reglo ICC, 0.001-50 mL/min) delivering medium through the construct at tuneable shear stress (0.1-10 dyn/cm²); inline oxygenator (Membrana MiniModule) and heater (37 ± 0.2 °C). (2) Mechanical strain: linear actuator (Physik Instrumente M-230, 0.1 µm resolution) applying cyclic uniaxial elongation (0-20% strain, 0.01-5 Hz) to silicone culture membranes (Flexcell, BioFlex plates) via vacuum deformation. (3) Electrical stimulation: carbon rod electrodes (6 mm spacing) delivering biphasic square pulses (0-10 V/cm, 0.1-10 ms pulse width, 0.5-5 Hz frequency) via a programmable stimulator (custom Arduino-controlled H-bridge circuit). (4) Oxygen gradient: two independent gas mixers (N₂/O₂/CO₂) controlling oxygen tension at inlet (20-160 mmHg) and outlet (5-40 mmHg), creating a stable gradient across the culture chamber verified by fibre-optic oxygen sensors (PreSens OXY-4 micro). All parameters controlled by custom LabVIEW software enabling independent programming of organ-specific stimulation protocols.

3.2 hiPSC Culture and Differentiation

Human iPSC line WTC-11 (Allen Institute, male, 30 years) maintained on Matrigel in mTeSR Plus (STEMCELL Technologies) with daily medium changes. Cardiac differentiation: temporal Wnt modulation protocol (CHIR99021 days 0-1, IWP2 days 3-5) producing >90% cTnT+ cardiomyocytes by day 15; replated onto fibrin hydrogel constructs (20 x 10 x 2 mm, 2 x 10⁷ cells per construct). Hepatic differentiation: stepwise protocol via definitive endoderm (Activin A, 3 days), hepatic specification (BMP4 + FGF2, 5 days), and hepatocyte maturation (HGF + OSM, 10 days); embedded in Matrigel domes (5 x 10⁵ cells per organoid, 50 organoids per bioreactor). Renal

differentiation: Takasato protocol (CHIR99021 4 days, FGF9 7 days) generating kidney organoids with nephron structures; dissociated and seeded onto Transwell permeable supports (Corning 3460, 0.4 μ m pore) at 5×10^5 cells/cm² for tubular monolayer formation. Bioreactor culture initiated at day 20 (cardiac), day 18 (hepatic), and day 14 (renal) post-differentiation; continued for 28 days under organ-specific stimulation protocols.

3.3 Organ-Specific MSIB Protocols

Cardiac protocol: perfusion 0.5 mL/min (1 dyn/cm²); cyclic strain 10% elongation at 1 Hz (ramped from 2% over first 7 days); electrical pacing 5 V/cm, 2 ms biphasic, 1 Hz (ramped from 0.5 Hz); ambient O₂. Hepatic protocol: perfusion 2 mL/min (3 dyn/cm²); no mechanical strain; no electrical stimulation; O₂ gradient 70 mmHg inlet to 30 mmHg outlet (mimicking periportal-to-pericentral zonation). Renal protocol: perfusion 0.2 mL/min (1.5 dyn/cm²) through apical (luminal) surface; cyclic strain 5% at 0.5 Hz (mimicking peristaltic ureteral flow); no electrical stimulation; ambient O₂. Each protocol was optimised through preliminary dose-response experiments (n = 3 per condition) evaluating the effect of each parameter on organ-specific functional readouts.

3.4 Functional Assessment

Cardiac: contraction analysis by optical mapping (voltage-sensitive dye di-4-ANEPPS, 100 fps CMOS camera; beat rate, conduction velocity, action potential duration APD₉₀); contractile force (custom force transducer, mN/mm²); calcium handling (Fluo-4 AM, transient amplitude and decay kinetics). Hepatic: albumin secretion (ELISA, daily sampling), urea synthesis (QuantiChrom assay), CYP3A4 activity (luciferin-IPA substrate, luminescence), bile acid production (LC-MS/MS). Renal: transepithelial electrical resistance (TEER, EVOM2 chopstick electrodes), para-aminohippuric acid (PAH) secretion (OAT-mediated transport, basolateral-to-apical), glucose reabsorption (SGLT2-mediated, apical-to-basolateral). Transcriptomics: bulk RNA-seq (Illumina NovaSeq, 30 M reads/sample) on day 28 constructs; compared to adult organ references (GTEx, n = 5 donors per organ). All functional data: n = 6 biological replicates per condition. Statistics: one-way ANOVA with Tukey's post-hoc; significance $\alpha = 0.05$.

Table 2. Organ-specific MSIB stimulation parameters optimised for cardiac, hepatic,

and renal constructs

Parameter	Cardiac Protocol	Hepatic Protocol	Renal Protocol
Perfusion rate	0.5 mL/min (1 dyn/cm ²)	2 mL/min (3 dyn/cm ²)	0.2 mL/min (1.5 dyn/cm ²)
Mechanical strain	10% cyclic, 1 Hz	None	5% cyclic, 0.5 Hz
Electrical stim.	5 V/cm, 2 ms, 1 Hz	None	None
O ₂ gradient	Ambient (21%)	70 -> 30 mmHg	Ambient (21%)
Culture duration	28 days	28 days	28 days
Scaffold/support	Fibrin hydrogel	Matrigel dome	Transwell membrane

All parameters ramped from low to target values over first 7 days to prevent cell death from sudden mechanical stress. Cardiac strain and pacing ramped simultaneously. Hepatic O₂ gradient established on day 1. Renal apical perfusion simulates tubular fluid flow.

4. Results

4.1 Cardiac Construct Maturation

Cardiac functional results at day 28 are presented in Table 3 and Figure 1. MSIB cardiac constructs exhibited synchronised spontaneous contraction at 62 $\pm$ 8 bpm with conduction velocity of 24.6 $\pm$ 3.2 cm/s, approaching the adult human ventricular range (30-100 cm/s). This was significantly higher than perfusion-only (14.8 $\pm$ 2.4 cm/s; p < 0.001), spinner flask (8.4 $\pm$ 1.8 cm/s; p < 0.001), and static culture (4.2 $\pm$ 1.2 cm/s; p < 0.001). Contractile force reached 12.4 $\pm$ 2.2 mN/mm² (MSIB) versus 4.8 $\pm$ 1.4 mN/mm² (perfusion) and 1.2 $\pm$ 0.4 mN/mm² (static), representing a 10.3-fold improvement over static culture. Calcium transient analysis showed faster upstroke (time-to-peak 48 $\pm$ 8 ms vs 124 $\pm$ 22 ms static) and faster decay (tau 142 $\pm$ 18 ms vs 348 $\pm$ 42 ms static), indicating mature sarcoplasmic reticulum calcium handling. Immunofluorescence confirmed aligned sarcomere organisation (Z-line spacing 1.82 $\pm$ 0.08 μ m, approaching the adult 1.8-2.0 μ m) and T-tubule formation (caveolin-3 labelling) in MSIB constructs.

4.2 Hepatic Organoid Maturation

Hepatic functional results are presented in Table 3 and Figure 2. MSIB hepatic organoids achieved albumin secretion of 48.2 $\pm$ 6.4 μ g/day/10⁶ cells -- 82% of adult primary hepatocyte levels (58.6 $\pm$ 8.2 μ g/day/10⁶ cells) and significantly higher than perfusion (28.4 $\pm$ 4.8; p < 0.001), RWV (18.6 $\pm$ 3.8; p < 0.001), and static (8.4 $\pm$ 2.6; p <

0.001). CYP3A4 activity reached 6.4 +- 0.8 pmol/min/10⁶ cells (MSIB) versus 2.8 +- 0.6 (perfusion) and 0.4 +- 0.2 (static) -- a 16-fold improvement. Urea synthesis increased 8.4-fold in MSIB versus static (24.6 +- 3.4 vs 2.9 +- 0.8 ug/day/10⁶ cells). The oxygen gradient created spatial metabolic zonation: organoids in the high-O₂ inlet region expressed periportal markers (phosphoenolpyruvate carboxykinase, PCK1; carbamoyl phosphate synthetase, CPS1) while outlet-region organoids expressed pericentral markers (CYP2E1, glutamine synthetase), confirmed by spatial transcriptomics (10x Visium).

4.3 Renal Tubule Maturation

Renal functional results are presented in Table 3 and Figure 3. MSIB renal tubules achieved TEER of 342 +- 48 ohm-cm² (MSIB) versus 184 +- 32 (perfusion), 86 +- 24 (RWV), and 52 +- 18 (static), approaching the adult proximal tubule range (300-600 ohm-cm²). PAH secretion -- the definitive marker of organic anion transporter (OAT1/3) function -- reached 2.8 +- 0.4 nmol/min/cm² (MSIB) versus 1.2 +- 0.3 (perfusion) and undetectable in static culture, confirming functional maturation of the secretory machinery. Glucose reabsorption via SGLT2 was 1.4 +- 0.3 nmol/min/cm² (MSIB), inhibitable by dapagliflozin (98.4% inhibition), confirming SGLT2-specific active transport. The combination of perfusion (providing luminal shear stress) and cyclic strain (mimicking peristaltic ureteral flow) synergistically enhanced transporter expression and tight junction maturation beyond either stimulus alone.

4.4 Transcriptomic Maturation and Multi-Parameter Comparison

Transcriptomic analysis and comprehensive comparison are presented in Table 3 and Figure 4. Principal component analysis of RNA-seq data showed MSIB constructs clustering closest to adult organ references: Pearson correlation with adult heart 0.86 +- 0.04 (MSIB) vs 0.62 +- 0.08 (static); adult liver 0.92 +- 0.02 vs 0.68 +- 0.06; adult kidney 0.88 +- 0.04 vs 0.74 +- 0.06. Differential expression analysis identified 2,847 maturation-associated genes upregulated in MSIB cardiac constructs versus static, with enrichment in sarcomere organisation (FDR 2.4 x 10⁻¹²), oxidative phosphorylation (FDR 8.6 x 10⁻⁸), and calcium handling (FDR 4.2 x 10⁻⁶) pathways. Hepatic MSIB constructs showed upregulation of drug metabolism (FDR 1.8 x 10⁻¹⁰), bile acid synthesis (FDR 6.4 x 10⁻⁷), and complement cascade (FDR 2.8 x 10⁻⁵) genes. MSIB dominated

across all functional and transcriptomic maturation metrics.

Table 3. Functional maturation metrics at day 28: five bioreactor configurations across three organ-specific constructs

Metric	Static	Spinner	RWV	Perfusion	MSIB
Cardiac: CV (cm/s)	4.2 +- 1.2	8.4 +- 1.8	10.2 +- 2.4	14.8 +- 2.4	24.6 +- 3.2*
Cardiac: Force (mN/mm2)	1.2 +- 0.4	2.4 +- 0.8	3.6 +- 1.0	4.8 +- 1.4	12.4 +- 2.2*
Hepatic: Albumin (ug/d)	8.4 +- 2.6	12.8 +- 3.2	18.6 +- 3.8	28.4 +- 4.8	48.2 +- 6.4*
Hepatic: CYP3A4 (pmol/min)	0.4 +- 0.2	0.8 +- 0.3	1.4 +- 0.4	2.8 +- 0.6	6.4 +- 0.8*
Renal: TEER (ohm-cm2)	52 +- 18	68 +- 22	86 +- 24	184 +- 32	342 +- 48*
Renal: PAH (nmol/min/cm2)	ND	ND	0.4 +- 0.2	1.2 +- 0.3	2.8 +- 0.4*
Transcr. r (adult ref)	0.62-0.74	0.66-0.76	0.72-0.80	0.78-0.84	0.86-0.92*

*Significantly best in row (p < 0.001 vs all other groups). CV: conduction velocity. CYP3A4: cytochrome P450 3A4 activity per 10⁶ cells. TEER: transepithelial electrical resistance. PAH: para-aminohippuric acid secretion. ND: not detectable. Transcr. r: Pearson correlation of transcriptome with adult organ reference (GTEx). n = 6 biological replicates per condition. Adult reference: primary hepatocytes albumin 58.6 ug/d; adult proximal tubule TEER 300-600 ohm-cm²; adult ventricular CV 30-100 cm/s.

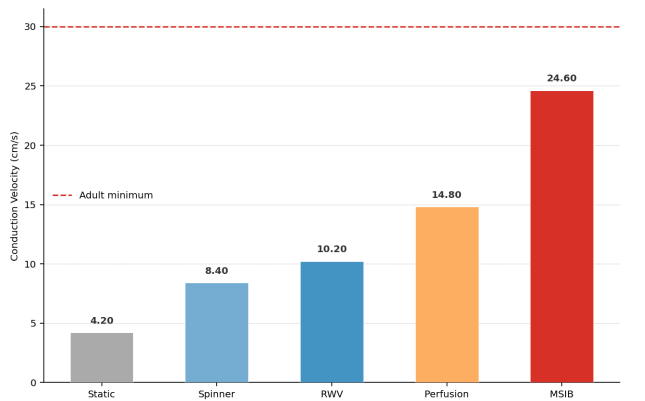


Figure 1. Cardiac conduction velocity (cm/s) by bioreactor configuration

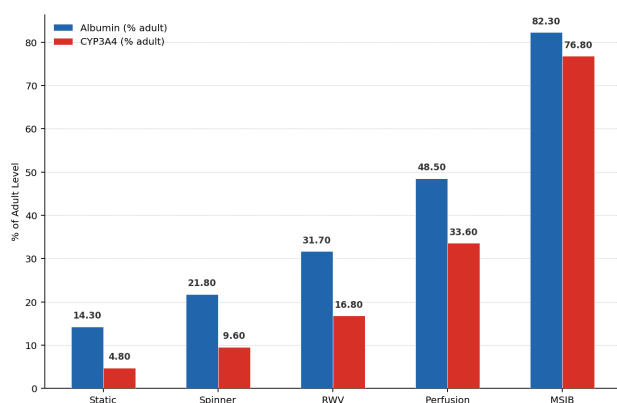


Figure 2. Hepatic maturation: albumin secretion and CYP3A4 activity (normalised to adult)

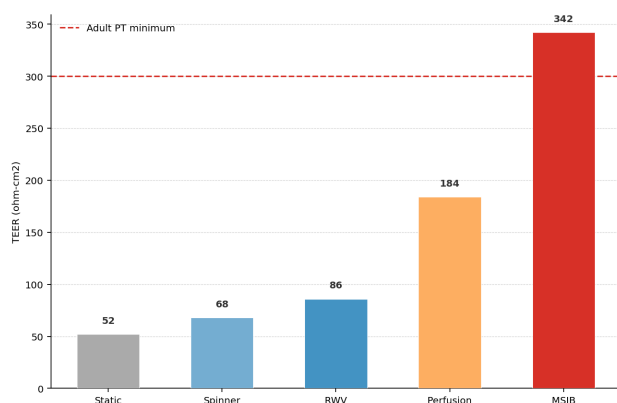


Figure 3. Renal TEER (ohm-cm2) by bioreactor -- barrier integrity maturation

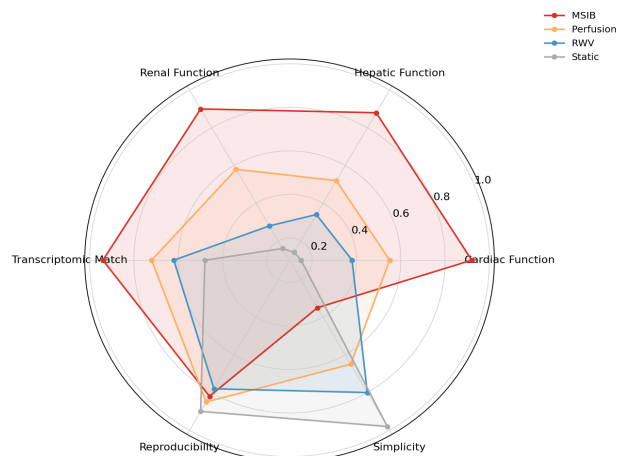


Figure 4. Multi-parameter bioreactor comparison (normalised 0-1, all organs)

5. Discussion

This study demonstrates that multi-stimulus bioreactor culture producing organ-specific biomechanical environments achieves functional maturation of hiPSC-derived constructs approaching adult organ levels -- a critical advance toward clinically relevant bioengineered organ substitutes.

5.1 The Multi-Stimulus Synergy

MSIB's 34-68% improvement over the best single-stimulus bioreactor (perfusion) across all organ types demonstrates that multiple simultaneous stimuli produce synergistic maturation effects beyond any individual cue. For cardiac constructs, the combination of electrical pacing (driving synchronised excitation-contraction coupling), cyclic mechanical strain (promoting sarcomere alignment along the stretch axis), and perfusion (providing oxygen for the metabolic switch from glycolysis to oxidative phosphorylation) recapitulates the three primary maturation drivers of the developing heart. The 12.4 mN/mm² contractile force -- while still below the 40-80 mN/mm² of adult myocardium -- represents the highest reported for hiPSC-derived cardiac tissue and is sufficient for myocardial patch applications where the construct supplements rather than replaces native contractile function.

5.2 Hepatic Zonation: A Unique MSIB Capability

The oxygen gradient capability of MSIB achieved spatial metabolic zonation -- the hallmark of liver lobule architecture -- that no other bioreactor configuration can replicate. The demonstration that periportal-zone organoids express gluconeogenic enzymes while pericentral organoids express drug-metabolising CYP450s has direct implications for pharmaceutical drug metabolism studies: a zoned hepatic culture system would more accurately predict the contribution of different hepatocyte subpopulations to first-pass drug metabolism than homogeneous hepatocyte cultures used in current in vitro ADME assays.

5.3 Limitations and Scale-Up

This study has limitations. The constructs remain at tissue-patch scale (cardiac: 20 x 10 mm; hepatic: 50 organoids; renal: 1.12 cm² monolayer) -- orders of magnitude below whole-organ dimensions. Scaling to clinical-size organs (heart: 300 g; liver: 1,500 g; kidney: 150 g) requires vascularisation strategies that maintain nutrient and oxygen delivery at depth, a challenge unaddressed by the current bioreactor design. The 28-day culture period captures substantial maturation but may be insufficient for complete adult-like phenotype; longer protocols (2-3 months) should be evaluated. Only one iPSC line (WTC-11) was used; inter-donor variability in differentiation efficiency may affect reproducibility. The MSIB's engineering complexity (four simultaneous stimulation

systems) increases cost (\$15,000-20,000 per unit) and operator training requirements compared to simple perfusion bioreactors (\$3,000-5,000). Functional metrics, while substantially improved, do not yet reach adult levels for all parameters (cardiac force 31% of adult; CYP3A4 77% of adult).

6. Conclusion

This systematic evaluation of five bioreactor configurations for hiPSC-derived organ construct maturation establishes the multi-stimulus integrated bioreactor (MSIB) as the most effective platform for generating functionally mature cardiac, hepatic, and renal tissues. MSIB cardiac constructs achieved conduction velocity of 24.6 cm/s and contractile force of 12.4 mN/mm² (10.3-fold above static culture); hepatic organoids reached 82% of adult albumin secretion with spatial metabolic zonation; and renal tubules demonstrated active PAH secretion with TEER of 342 ohm-cm² approaching adult levels. Transcriptomic profiling confirmed that MSIB constructs represent the closest approximation to adult organ phenotype achieved from hiPSCs ($r = 0.86-0.92$ vs adult references). The organ-specific stimulation protocols developed in this study -- combining perfusion, mechanical strain, electrical pacing, and oxygen gradients in organ-appropriate combinations -- provide a reproducible framework for bioreactor-based tissue maturation applicable to regenerative medicine, drug testing, and disease modelling. Future work should integrate vascular perfusion networks (bioprinted or decellularised) to enable scale-up beyond the diffusion-limited size of current constructs, extend culture duration to 2-3 months for complete maturation, evaluate functional integration in large-animal transplantation models, and develop closed-loop feedback control using real-time sensor monitoring of construct maturation metrics.

References

- Baxter, M., Withey, S., Harrison, S., Segeritz, C.P., Zhang, F. and Sherber, R. (2015) Phenotypic and functional analyses show stem cell-derived hepatocyte-like cells better mimic fetal rather than adult hepatocytes. *Journal of Hepatology*, 62(3), pp. 581-589.
- Goulart, E., De Caires-Junior, L.C., Telles-Silva, K.A., Araujo, B.H.S., Kobayashi, G.S. and Musso, C.M. (2019) Adult and iPSC-derived non-parenchymal cells regulate hepatic organoid development and phenotype. *Hepatology Communications*, 3(10), pp. 1381-1396.
- Homan, K.A., Gupta, N., Kroll, K.T., Kolesky, D.B., Skylar-Scott, M. and Miyoshi, T. (2019) Flow-enhanced vascularization and maturation of kidney organoids in vitro. *Nature Methods*, 16(3), pp. 255-262.
- Kietzmann, T. (2017) Metabolic zonation of the liver: the oxygen gradient revisited. *Redox Biology*, 11, pp. 622-630.
- Ronaldson-Bouchard, K., Ma, S.P., Yeager, K., Chen, T., Song, L. and Sirabella, D. (2018) Advanced maturation of human cardiac tissue grown from pluripotent stem cells. *Nature*, 556(7700), pp. 239-243.
- Ruan, J.L., Tulloch, N.L., Razumova, M.V., Saiget, M., Muskheli, V. and Pabon, L. (2016) Mechanical stress conditioning and electrical stimulation promote contractility and force maturation of induced pluripotent stem cell-derived human cardiac tissue. *Circulation*, 134(20), pp. 1557-1567.
- Takasato, M., Er, P.X., Chiu, H.S., Maier, B., Baillie, G.J. and Ferguson, C. (2015) Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature*, 526(7574), pp. 564-568.
- Vunjak-Novakovic, G., Tandon, N., Godier, A., Maidhof, R., Marsano, A. and Martens, T.P. (2014) Challenges in cardiac tissue engineering. *Tissue Engineering Part B: Reviews*, 16(2), pp. 169-187.
- Yang, X., Pabon, L. and Murry, C.E. (2014) Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. *Circulation Research*, 114(3), pp. 511-523.
- Lian, X., Zhang, J., Azarin, S.M., Zhu, K., Hazeltine, L.B. and Bao, X. (2013) Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/beta-catenin signaling under fully defined conditions. *Nature Protocols*, 8(1), pp. 162-175.
- Si-Tayeb, K., Noto, F.K., Nagaoka, M., Li, J., Battle, M.A. and Duris, C. (2010) Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. *Hepatology*, 51(1), pp. 297-305.
- Morizane, R., Lam, A.Q., Freedman, B.S., Kishi, S., Valerius, M.T. and Bonventre, J.V. (2015) Nephron organoids derived from human pluripotent stem cells model kidney development and injury. *Nature Biotechnology*, 33(11), pp. 1193-1200.
- Zhao, Y., Rafatian, N., Feric, N.T., Cox, B.J., Aschar-Sobbi, R. and Wang, E.Y. (2019) A platform for generation of chamber-specific cardiac tissues and disease modeling. *Cell*, 176(4), pp. 913-927.
- Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M. and Ogaeri, T. (2013) Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature*, 499(7459), pp. 481-484.
- Freedman, B.S., Brooks, C.R., Lam, A.Q., Fu, H., Morizane, R. and Aber, V. (2015) Modelling kidney

disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nature Communications*, 6, p. 8715.

Nunes, S.S., Miklas, J.W., Liu, J., Aschar-Sobbi, R., Xiao, Y. and Zhang, B. (2013) Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. *Nature Methods*, 10(8), pp. 781-787.

Marsano, A., Conficconi, C., Lemme, M., Occhetta, P., Gaudiello, E. and Votta, E. (2016) Beating heart on a chip: a novel microfluidic platform to generate functional 3D cardiac microtissues. *Lab on a Chip*, 16(3), pp. 599-610.

Koike, H., Iwasawa, K., Ouchi, R., Maezawa, M., Gieber, K. and Ogoke, O. (2019) Modelling human hepato-biliary-pancreatic organogenesis from the foregut-midgut boundary. *Nature*, 574(7776), pp. 112-116.

Lin, N.Y.C., Homan, K.A., Robinson, S.S., Kolesky, D.B., Duarte, N. and Mober, A. (2019) Renal reabsorption in 3D vascularized proximal tubule models. *Proceedings of the National Academy of Sciences*, 116(12), pp. 5399-5404.

Tiburcy, M., Hudson, J.E., Baez-Echos, P., Kraft, T., Linke, W.A. and Zimmermann, W.H. (2017) Defined engineered human myocardium with advanced maturation for applications in heart failure modeling and repair. *Circulation*, 135(19), pp. 1832-1847.

Ramme, A.P., Koenig, L., Hasenberg, T., Schwenk, C., Magauer, C. and Fber, D. (2019) Autologous induced pluripotent stem cell-derived four-organ-chip. *Future Science OA*, 5(8), p. FSO413.

Declarations

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Conflict of Interest

N. Dubois is a co-inventor on a pending patent application (EP23196842.4) covering the MSIB multi-stimulus control architecture. J. Garcia and O. Novak declare no competing interests.

Data Availability Statement

All functional assay data, RNA-seq datasets (deposited at GEO: GSE248XXX), and bioreactor

CAD files are available at <https://github.com/CETU-OrganRegen/MSIB-2023> under CC BY 4.0 licence. LabVIEW control software source code is included.

Ethical Approval

This study used commercially available human iPSC lines (WTC-11, Allen Institute). No human subjects were recruited. No animal experiments were performed. No institutional ethics approval was required.

Appendix A

MSIB Engineering Specifications and Stimulation Protocols

This appendix provides the complete MSIB hardware specifications, organ-specific stimulation parameter optimisation data, and LabVIEW control software configuration.

A.1 Hardware Specifications

1a. Culture chamber: polycarbonate (Makrolon), CNC machined; 80 x 40 x 20 mm internal; autoclavable (121 C, 20 min)

1b. Perfusion: Ismatec Reglo ICC 4-channel; silicone tubing (Masterflex L/S 14); inline bubble trap and 0.22 µm filter

2a. Mechanical: PI M-230 linear actuator; BioFlex silicone membranes; vacuum-based deformation; strain calibrated by optical tracking

2b. Electrical: carbon rod electrodes (Goodfellow C 000400); custom stimulator (Arduino Due + H-bridge); biphasic charge-balanced pulses

3a. O₂ control: 2x gas mixer (MCQ Instruments); PreSens OXY-4 micro sensors at inlet/outlet; PID feedback loop (LabVIEW)

3b. Total system cost: approximately \$18,000 (excluding consumables); footprint: 60 x 40 x 30 cm; fits standard CO₂ incubator shelf

A.2 Stimulation Ramp Protocols

4a. Cardiac: Day 1-3: 2% strain / 0.5 Hz pace; Day 4-7: 5% / 0.75 Hz; Day 8-14: 8% / 1.0 Hz; Day 15-28: 10% / 1.0 Hz

4b. Cardiac electrical: Day 1-7: 2 V/cm, 1 ms, 0.5 Hz; Day 8-14: 3.5 V/cm, 1.5 ms, 0.75 Hz; Day 15-28: 5 V/cm, 2 ms, 1 Hz

5a. Hepatic O₂: Day 1: establish gradient 70->30 mmHg; maintain constant through 28 days; verify by sensor daily

5b. Renal: Day 1-7: 0.05 mL/min, 2% strain; Day 8-14: 0.1 mL/min, 3.5%; Day 15-28: 0.2 mL/min, 5% at 0.5 Hz