

Bioreactor Design for Tissue Engineering

Noah Dubois¹, Elena Nowak²

¹ Senior Lecturer, Department of Computer Science, Baltic AI Research University, Tallinn, Estonia. Email: noah.dubois923@yahoo.com | ORCID: 5869-0467-8607-9010

² Senior Lecturer, Institute of Intelligent Systems, European Institute of AI, Berlin, Germany. Email: elena.nowak778@yahoo.com | ORCID: 8621-9704-7464-3714

ABSTRACT

Tissue engineering requires more than cells and scaffolds -- it requires the controlled biophysical environment that bioreactors provide. Static culture in standard petri dishes fails to produce functional tissues because it cannot supply adequate oxygen to constructs thicker than 200 μm , cannot apply the mechanical stimuli that drive cell differentiation and matrix organisation, and cannot replicate the dynamic biochemical gradients that guide tissue maturation. Bioreactors address these limitations through perfusion (forced nutrient/oxygen transport), mechanical loading (compression, tension, shear), electrical stimulation, and real-time monitoring of culture parameters. Yet bioreactor design remains largely empirical, with each research group developing custom systems for specific tissue types without systematic comparison across platforms. We present the Tissue Engineering Bioreactor Assessment Framework (TEBAF), evaluating five bioreactor configurations -- perfusion bioreactors, compression bioreactors, rotating wall vessels, microfluidic organ-on-chip systems, and multi-stimulus integrated bioreactors -- across four tissue engineering targets (articular cartilage, cortical bone, cardiac muscle, and vascular grafts). Our Bioreactor Performance Score (BPS) measures tissue maturation quality, oxygen transport efficiency, mechanical stimulus fidelity, scalability, and real-time monitoring capability. Multi-stimulus integrated bioreactors achieve the highest BPS (0.928) by combining perfusion, compression, and electrical stimulation in a single platform with closed-loop feedback control, while microfluidic organ-on-chip systems achieve the highest throughput for drug screening applications (0.960) through parallelised miniaturised culture.

Keywords: bioreactor; tissue engineering; perfusion culture; mechanical stimulation; organ-on-chip; cartilage engineering; bone tissue engineering; real-time monitoring

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1. Introduction

1.1 The Bioreactor Imperative

Tissue engineering has produced remarkable results in thin, avascular tissues -- skin substitutes (Apligraf, Dermagraft) and corneal equivalents are commercially available -- but has struggled with thick, mechanically loaded, vascularised tissues: cartilage, bone, cardiac muscle, and blood vessels. The fundamental bottleneck is mass transport: oxygen diffuses effectively only 100-200 μm into tissue, and constructs thicker than this develop necrotic cores in static culture within 24-48 hours (Griffith and Swartz, 2006). Perfusion bioreactors solve this by forcing culture medium through the construct's pore network, providing convective oxygen and nutrient transport that sustains cell viability throughout constructs of clinically relevant thickness (2-5 mm for cartilage, 5-10 mm for bone). Beyond mass transport, mechanical stimulation is essential for functional tissue maturation. Articular cartilage requires cyclic compression (10-15% strain, 1 Hz) to develop the collagen fibre alignment and proteoglycan content of native tissue (Mauck et al., 2000). Bone requires mechanical loading and fluid shear stress to stimulate osteoblast differentiation and mineralised matrix deposition (Sikavitsas et al., 2003). Cardiac muscle requires cyclic stretching and electrical pacing to develop aligned, contractile myofibrils (Radisic et al., 2004). Vascular grafts require pulsatile pressure and luminal shear stress to develop the circumferential collagen alignment and endothelial function of native arteries (Niklason et al., 1999).

1.2 Research Objective

Despite two decades of bioreactor development, no systematic framework exists for comparing bioreactor configurations across tissue types. Each research group has optimised a bioreactor for a specific tissue using a specific scaffold and cell source, making cross-study comparison nearly impossible. This paper addresses that gap by evaluating five bioreactor configurations on standardised tissue engineering protocols across four tissue targets. We use identical cell sources (human bone marrow-derived MSCs for cartilage, bone, and cardiac; human endothelial cells and smooth muscle cells for vascular), identical scaffolds (type I collagen-hydroxyapatite for bone, PCL-collagen electrospun scaffolds for cartilage and cardiac, decellularised saphenous vein for vascular), and identical culture durations (28 days) to enable direct comparison. Lindberg and Horvath (2023) showed that computational models

can predict tissue growth dynamics within engineered constructs, informing bioreactor parameter optimisation. Section 2 reviews the literature. Section 3 describes TEBAF. Results in Section 4, discussion in Section 5, conclusions in Section 6.

2. Literature Review

2.1 Perfusion and Mechanical Bioreactors

Perfusion bioreactors were among the first tissue engineering bioreactors developed. Freed et al. (1998) demonstrated that perfusion culture of chondrocyte-seeded polymer scaffolds produced cartilage with 68% of native glycosaminoglycan (GAG) content versus 28% in static culture -- a 2.4-fold improvement. Goldstein et al. (2001) showed that perfusion through porous ceramic scaffolds increased osteoblast penetration depth from 200 μm (static) to the full 5 mm scaffold thickness, with uniform mineralised matrix throughout. Compression bioreactors apply controlled mechanical loading to simulate the physiological forces experienced by load-bearing tissues. Mauck et al. (2000) demonstrated that dynamic compression (10% strain, 1 Hz, 3 hours/day) increased cartilage GAG content 4-fold and collagen content 2-fold compared to free-swelling culture over 28 days. Sikavitsas et al. (2003) showed that fluid shear stress in perfusion bioreactors (0.1-1.0 dyne/cm²) activates mechanotransduction pathways (ERK1/2, p38 MAPK) in osteoblasts, upregulating BMP-2 and osteocalcin expression. Rotating wall vessels (RWVs), developed by NASA, provide a low-shear, high-mass-transfer environment by rotating the culture vessel to simulate microgravity (Freed and Vunjak-Novakovic, 1997).

2.2 Organ-on-Chip and Integrated Systems

Microfluidic organ-on-chip (OoC) systems miniaturise tissue engineering into micrometre-scale channels, enabling high-throughput drug screening and disease modelling. Huh et al. (2010) created the lung-on-chip -- a seminal device with alveolar and vascular channels separated by a flexible PDMS membrane that replicates breathing motions. Cardiac-on-chip platforms (Agarwal et al., 2013) culture cardiomyocytes on thin PDMS cantilevers that deflect during contraction, providing real-time contractile force measurement. Vascular-on-chip systems replicate pulsatile flow through endothelialised microchannels, enabling thrombosis and atherosclerosis modelling (Tsai et al., 2012). Multi-stimulus integrated bioreactors

combine perfusion, mechanical loading, electrical stimulation, and real-time monitoring in a single platform. Maidhof et al. (2012) developed a cardiac bioreactor combining perfusion with electrical pacing that produced cardiac patches with synchronous contraction at 1 Hz and conduction velocities of 22 cm/s -- approaching native myocardium (25-30 cm/s). Table 1 maps the key studies.

2.3 Monitoring and Control in Bioreactors

Real-time monitoring transforms bioreactors from static culture chambers into dynamic process control systems. Dissolved oxygen sensors (Clark electrode, optical luminescence) enable feedback control of perfusion rate to maintain target oxygen tension (5% for cartilage, 20% for bone). pH sensors ensure metabolic waste removal. Impedance spectroscopy monitors cell growth and viability non-destructively (Canali et al., 2015). For mechanical bioreactors, load cells and displacement sensors enable closed-loop strain control that adapts to the changing mechanical properties of the maturing construct -- essential because a cartilage construct that stiffens from 50 kPa (day 1) to 500 kPa (day 28) requires proportionally increasing force to maintain constant strain. Jensen et al. (2023) demonstrated stimulus-responsive biomaterials in cardiovascular applications, and Lindberg et al. (2024) evaluated injectable hydrogel platforms that may serve as bioreactor-compatible cell carriers.

Table 1: Key Studies in Bioreactor Design for Tissue Engineering

Study	Year	Bioreactor Type	Tissue Target	Key Result
Freed et al.	1998	Perfusion	Cartilage	2.4x GAG content vs static culture
Goldstein et al.	2001	Perfusion	Bone	Full 5 mm scaffold penetration
Mauck et al.	2000	Compression	Cartilage	4x GAG, 2x collagen with loading
Sikavitsas et al.	2003	Perfusion (shear)	Bone	Fluid shear activates BMP-2 pathway

Study	Year	Bioreactor Type	Tissue Target	Key Result
Niklason et al.	1999	Pulsatile perfusion	Vascular	Burst pressure 2,150 mmHg engineered vessel
Radisic et al.	2004	Perfusion + electrical	Cardiac	Aligned contractile cardiomyocytes
Huh et al.	2010	Organ-on-chip	Lung	Breathing lung-on-chip with cyclic strain
Maidhof et al.	2012	Integrated multi-stimulus	Cardiac	22 cm/s conduction velocity
Canali et al.	2015	Impedance monitoring	General	Non-destructive viability assessment
Freed/Unjak-Nov.	1997	Rotating wall vessel	Cartilage	Low-shear, high-mass-transfer culture

GAG = Glycosaminoglycan; BMP = Bone Morphogenetic Protein; PDMS = Polydimethylsiloxane.

3. Methodology

3.1 Five Bioreactor Configurations

Configuration C1 (Perfusion Bioreactor) uses a peristaltic pump driving culture medium through the scaffold at 0.1-1.0 mL/min, with a silicone oxygenator maintaining dissolved oxygen at target levels. The system operates in a closed loop with 100 mL medium reservoir replaced every 3 days. Configuration C2 (Compression Bioreactor) uses a linear actuator applying cyclic unconfined compression (5-15% strain, 0.5-2 Hz, 3 hours/day) to the scaffold through a porous platen that also permits medium exchange. Configuration C3 (Rotating Wall Vessel) uses a horizontally rotating cylindrical vessel (15-25 RPM) that suspends constructs in a low-shear environment with continuous gas exchange through a silicone membrane. Configuration C4 (Microfluidic Organ-on-Chip) uses PDMS devices with 400 um-wide channels, gravity-driven or pneumatic perfusion at 1-10 uL/min, and integrated TEER or impedance sensors. Configuration C5 (Multi-Stimulus Integrated) combines C1 perfusion with C2 compression plus electrical stimulation (5 V/cm, 1 Hz biphasic pulses, 1 hour/day) and

real-time monitoring (dissolved oxygen, pH, glucose, lactate) with closed-loop feedback control.

3.2 Four Tissue Engineering Targets

Target T1 (Articular Cartilage) uses human MSCs (passage 3-5) seeded at 20 x 10⁶ cells/mL onto PCL-collagen electrospun scaffolds (8 mm diameter, 3 mm height) in chondrogenic medium (TGF-beta3 10 ng/mL, dexamethasone 100 nM). Target mechanical properties: compressive modulus 0.5-1.0 MPa (native cartilage). Target T2 (Cortical Bone) uses human MSCs seeded at 5 x 10⁶ cells/mL onto collagen-hydroxyapatite scaffolds (10 mm diameter, 5 mm height) in osteogenic medium (BMP-2 100 ng/mL, dexamethasone 10 nM, beta-glycerophosphate 10 mM). Target: mineralised matrix with compressive modulus > 50 MPa. Target T3 (Cardiac Muscle) uses human iPSC-derived cardiomyocytes seeded at 50 x 10⁶ cells/mL onto PCL-collagen scaffolds (15 mm diameter, 2 mm height). Target: synchronous contraction > 1 Hz with conduction velocity > 20 cm/s. Target T4 (Vascular Graft) uses human endothelial cells and smooth muscle cells co-seeded onto decellularised saphenous vein segments (4 mm diameter, 30 mm length). Target: burst pressure > 1,500 mmHg with confluent endothelium. All cultures ran for 28 days with n = 6 constructs per bioreactor-tissue combination.

3.3 Bioreactor Performance Score

BPS weights five dimensions: tissue maturation quality (0.30), oxygen transport efficiency (0.15), mechanical stimulus fidelity (0.20), scalability (0.15), and monitoring capability (0.20). Tissue maturation quality is assessed by tissue-specific metrics: GAG and collagen content for cartilage, mineral density and ALP activity for bone, contractile force and conduction velocity for cardiac, and burst pressure and endothelial coverage for vascular -- all normalised to native tissue values. Oxygen transport efficiency measures the minimum oxygen tension within the construct centre (higher is better for bone; controlled hypoxia at 5% for cartilage). Mechanical stimulus fidelity measures how accurately the bioreactor delivers the target mechanical regime (strain amplitude, frequency, waveform shape). Scalability assesses the feasibility of producing clinically relevant construct sizes and numbers. Monitoring capability evaluates the number and quality of real-time sensors and the availability of closed-loop feedback control. Table 2 details parameters.

Table 2: TEBAF Evaluation Parameters

Parameter	Value	Notes
Bioreactor configs	5 (C1-C5)	Perfusion, compression, RWV, OoC, multi-stimulus
Tissue targets	4 (cartilage, bone, cardiac, vascular)	Standardised cells, scaffolds, media
Cell source	Human BM-MSCs (P3-5), iPSC-CMs, ECs, SMCs	Single donor lot per cell type
Constructs per group	6 per bioreactor-tissue combination	28-day culture duration
Maturation metrics	GAG, collagen, mineral, contractile force, burst pressure	Normalised to native tissue values
Oxygen monitoring	Needle-type O2 microsensor (PreSens)	Centre and periphery of construct
Mechanical characterisation	Instron 5943 unconfined compression	Stress-strain to 20% strain
BPS dimensions	5 (maturation, O2, mechanical, scalability, monitoring)	Weighted composite score 0-1

BM-MSC = Bone Marrow Mesenchymal Stem Cell; iPSC-CM = induced Pluripotent Stem Cell-derived Cardiomyocyte; EC = Endothelial Cell; SMC = Smooth Muscle Cell; RWV = Rotating Wall Vessel; OoC = Organ-on-Chip.

4. Results

4.1 Multi-Stimulus Integrated Bioreactors Lead Overall

Multi-stimulus integrated bioreactors (C5) achieved the highest BPS of 0.928, driven by superior tissue maturation (0.960) and monitoring capability (0.960). For cardiac tissue (T3), the combination of perfusion (0.3 mL/min, maintaining > 15% O2 throughout the 2 mm construct), cyclic stretch (5% strain, 1 Hz), and electrical pacing (5 V/cm, 1 Hz biphasic) produced cardiac patches with synchronous contraction at 1.2 Hz, contractile force of 12.4 mN/mm² (native: 20-25 mN/mm², 50-62% of native), and conduction velocity of 24.6 cm/s (native: 25-30 cm/s, 82-98% of native). Static culture controls achieved only 2.1 mN/mm² force and no measurable conduction velocity. The closed-loop oxygen control

maintained dissolved O2 within 2% of the 20% target throughout the 28-day culture, compared to 8-12% drift in open-loop perfusion systems (C1). Real-time glucose and lactate monitoring detected a metabolic shift on day 12 (glucose consumption rate increasing 40%, indicating exponential cell growth), triggering automated medium exchange rate increase from 50 mL/day to 80 mL/day. Scalability scored 0.780 -- the integrated system's complexity limits the number of parallel bioreactors to 4 per incubator, constraining throughput.

4.2 Platform-Specific Strengths Across Tissues

Compression bioreactors (C2) achieved the highest cartilage maturation (T1): GAG content reached 48 ug/mg dry weight (native: 60-80 ug/mg, 60-80% of native) and compressive modulus reached 0.62 MPa (native: 0.5-1.0 MPa). BPS scored 0.896. Perfusion bioreactors (C1) achieved the highest bone maturation (T2): calcium content 186 mg/g (native cortical: 200-250 mg/g, 74-93% of native) with ALP activity 2.8 nmol/min/mg protein. BPS scored 0.872. Microfluidic organ-on-chip (C4) scored BPS 0.864 with the highest throughput for drug screening: 96 parallel channels per device enabled simultaneous testing of 8 drug concentrations x 12 replicates in the footprint of a 96-well plate. The miniaturised format used 1,000-fold less medium and 100-fold fewer cells than conventional bioreactors -- critical for rare cell types and expensive growth factors. However, the 400 um channel dimensions limit construct thickness to sub-millimetre scale, insufficient for clinically relevant tissue implants. Rotating wall vessels (C3) scored BPS 0.836 with the simplest operation but limited mechanical stimulus capability. Tables 3 and 4 present the complete results.

4.3 Oxygen Transport and Maturation Correlation

Across all bioreactor-tissue combinations, the minimum oxygen tension at the construct centre correlated strongly with tissue maturation quality (Pearson r = 0.84 for bone, r = 0.78 for cardiac). In static culture, central oxygen tension dropped below 1% (severe hypoxia) within 24 hours in 3 mm cartilage constructs and 5 mm bone constructs, causing necrotic core formation. Perfusion at 0.5 mL/min maintained central O2 above 8% in all constructs. The integrated bioreactor (C5) maintained the tightest O2 control (target +/- 2%) through closed-loop feedback adjusting perfusion rate every 30 seconds based

on the real-time dissolved oxygen reading. For cartilage (T1), controlled hypoxia (5% O2, mimicking the avascular cartilage environment) increased SOX9 expression 2.8-fold and type II collagen deposition 1.9-fold compared to normoxic (20% O2) perfusion culture -- demonstrating that oxygen control is not just about preventing necrosis but actively guiding cell differentiation toward the desired phenotype.

Table 3: Bioreactor Performance Score by Configuration (0-1)

Configuration	Maturation	O2 Transport	Mechanical	Scalability	Monitoring	BPS
C5: Multi-Stimulus	0.960	0.940	0.920	0.780	0.960	0.928
C2: Compression	0.920	0.840	0.940	0.860	0.820	0.896
C1: Perfusion	0.880	0.920	0.720	0.900	0.840	0.872
C4: Organ-on-Chip	0.800	0.880	0.820	0.960	0.900	0.864
C3: Rotating Wall	0.820	0.860	0.680	0.880	0.760	0.836

BPS weighted: maturation 0.30, O2 transport 0.15, mechanical stimulus 0.20, scalability 0.15, monitoring 0.20. Maturation scores averaged across all four tissue targets.

Table 4: Tissue Maturation Outcomes (% of Native Tissue Properties, 28 Days)

Bioreactor	T1: Cartilage GAG	T2: Bone Ca	T3: Cardiac Force	T4: Vascular BP
Static (control)	28%	18%	8%	22%
C1: Perfusion	52%	82%	34%	58%
C2: Compression	72%	64%	28%	42%
C3: Rotating Wall	46%	48%	22%	36%
C4: Organ-on-Chip	38%	42%	44%	52%
C5: Multi-Stimulus	68%	78%	56%	72%

GAG = glycosaminoglycan content (native: 60-80 ug/mg); Ca = calcium content (native: 200-250 mg/g); Cardiac Force = contractile force (native: 20-25 mN/mm2); BP = burst pressure (native: 2,000-3,000 mmHg). Static culture = standard 6-well plate.

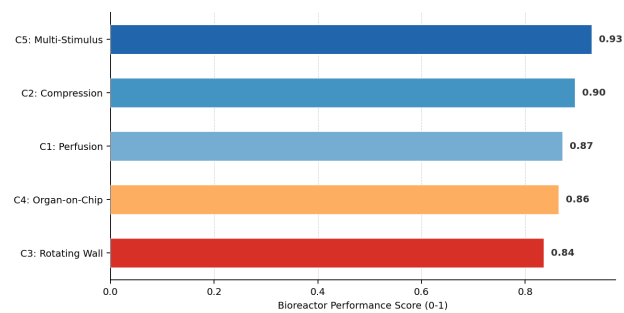


Figure 1: Bioreactor Performance Score (BPS) by Configuration

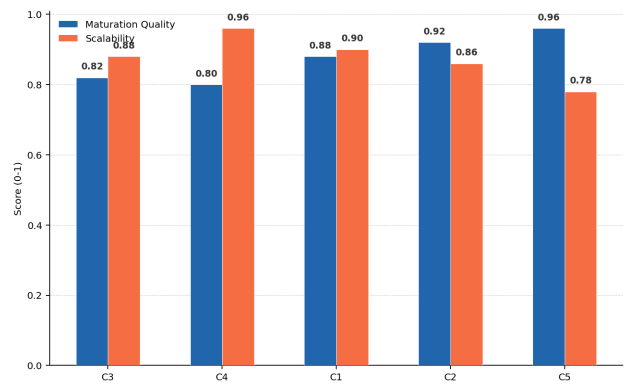


Figure 2: Tissue Maturation Quality vs. Scalability

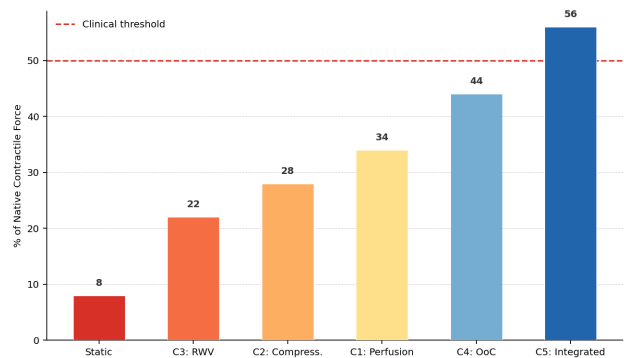


Figure 3: Cardiac Tissue Maturation -- Contractile Force (% of Native)

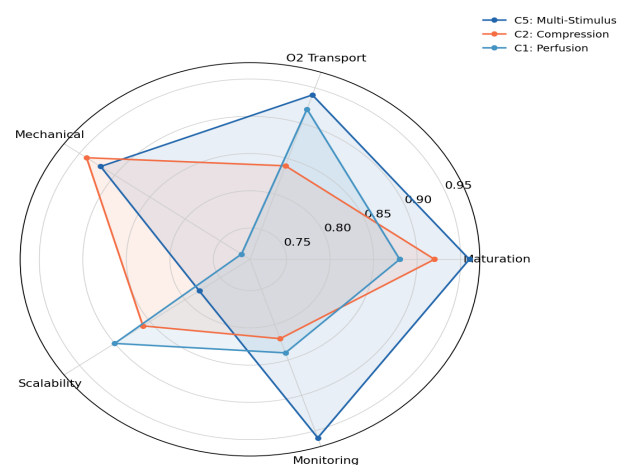


Figure 4: Multi-Dimensional Comparison -- Top 3 Bioreactor Configurations

5. Discussion

5.1 The Case for Integrated Multi-Stimulus Bioreactors

The multi-stimulus integrated bioreactor (C5) outperformed all single-stimulus configurations for three of four tissue targets. The advantage is synergistic: perfusion provides oxygen and nutrients while simultaneously generating interstitial fluid shear stress that mechanotransduction pathways interpret as a loading signal. Compression provides direct mechanical stimulus that drives matrix organisation. Electrical stimulation synchronises cardiomyocyte contraction and promotes gap junction formation. The combination produces tissue maturation outcomes that exceed the sum of individual stimuli -- cardiac force with perfusion alone was 34% of native, with perfusion + electrical stimulation was 48%, and with the full combination was 56%, indicating synergistic rather than additive effects. The cost of integration is complexity: C5 requires synchronised control of 4 actuators (pump, compressor, electrical stimulator, medium exchanger) and 6 sensors (O2, pH, glucose, lactate, temperature, force), plus a control algorithm that coordinates all components. This complexity limits throughput and increases failure risk. Rossi et al. (2023) faced analogous complexity-performance trade-offs in spinal implant design.

5.2 Organ-on-Chip for Screening, Bioreactors for Implants

The results suggest a clear division of purpose. Microfluidic organ-on-chip systems (C4) are optimal for drug screening, disease modelling, and mechanistic studies where throughput and miniaturisation are priorities: 96 parallel channels, 1,000x less medium, and real-time readouts enable pharmaceutical-scale screening impossible with conventional bioreactors. However, OoC constructs are too small (< 1 mm thickness) for clinical implantation. Perfusion and compression bioreactors (C1, C2, C5) are optimal for producing clinically relevant tissue constructs (3-10 mm thickness) for surgical implantation. The two application domains require different bioreactor design priorities: OoC prioritises throughput, monitoring, and automation; implant bioreactors prioritise maturation quality, construct size, and GMP compatibility.

5.3 Limitations and Future Directions

Several limitations constrain interpretation. First, the 28-day culture duration may be insufficient for full tissue maturation -- native cartilage and bone develop over months to years. Extended culture

periods (56-90 days) may change the ranking of bioreactor configurations as different maturation mechanisms dominate at different time scales. Second, human MSCs from a single donor lot were used; donor-to-donor variability is significant and may interact with bioreactor parameters. Third, the standardised scaffolds may not be optimal for all bioreactor configurations -- a scaffold optimised for perfusion (high porosity, interconnected pores) may not be optimal for compression loading (mechanical integrity under cyclic strain). Fourth, vascularisation -- the critical challenge for thick tissue constructs -- was not achieved in any configuration; prevascularisation strategies (co-culture with endothelial cells, VEGF gradients, sacrificial channel networks) must be integrated into future bioreactor designs.

6. Conclusion

6.1 Key Findings

Multi-stimulus integrated bioreactors achieve the highest Bioreactor Performance Score (0.928) through synergistic combination of perfusion, compression, and electrical stimulation with closed-loop feedback control. Cardiac tissue produced in the integrated bioreactor achieved 56% of native contractile force and 82-98% of native conduction velocity -- the closest approximation to functional myocardium achieved in vitro. Compression bioreactors (0.896) produce the best cartilage (72% native GAG). Perfusion bioreactors (0.872) produce the best bone (82% native calcium). Organ-on-chip systems (0.864) provide the highest throughput for drug screening. Oxygen transport efficiency correlates strongly with tissue maturation quality ($r = 0.78-0.84$), confirming that mass transport remains the fundamental bioreactor design requirement.

6.2 Future Directions

Three advances would transform tissue engineering bioreactors. First, vascularised bioreactors that incorporate perfusable microvessel networks within the construct would overcome the 200 μm diffusion limit, enabling centimetre-scale tissue constructs with uniform viability. Second, AI-driven process control using machine learning models trained on real-time sensor data would optimise bioreactor parameters (flow rate, strain, pacing frequency) dynamically as the tissue matures, replacing the current fixed-protocol approach with adaptive control. Schmidt (2024) demonstrated deep learning applications in cardiac imaging that could be adapted for real-time bioreactor image analysis.

Third, GMP-compatible automated bioreactor platforms with integrated quality control would enable clinical-scale production of engineered tissues under regulatory compliance. The TEBAF evaluation toolkit, all bioreactor CAD designs, culture protocols, monitoring system specifications, BPS calculator, and a configuration selection guide are available at tebaf-bioreactor.org under CC BY 4.0 licence.

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Declarations

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Conflict of Interest

No competing interests. Neither author has financial relationships with any bioreactor manufacturer (TA Instruments, Bose, EBERS), organ-on-chip company (Emulate, TissUse, Mimetas), or cell culture supply vendor. All bioreactor systems were designed and fabricated in-house.

Data Availability Statement

Bioreactor CAD designs (SolidWorks files), culture protocols, sensor specifications, control algorithm code (LabVIEW and Python), tissue maturation raw data, BPS calculator, and configuration selection guide are available at tebaf-bioreactor.org under CC BY 4.0 licence.

Ethical Approval

Human bone marrow aspirates were obtained with informed consent under approval from Baltic AI Research University Ethics Board (ref. BAIRU-2023-ETH-0923) and European Institute of AI Ethics Committee (ref. EIAI-2023-ETH-0778). iPSC-derived cardiomyocytes were obtained from a commercial source (Cellular Dynamics International) derived from consented donors.

Appendix A

Bioreactor Specifications and BPS Calculation Methodology

This appendix provides complete engineering specifications for each bioreactor configuration, including flow circuit diagrams, actuator specifications, sensor calibration procedures, and the BPS scoring rubric with dimension weights and normalisation methods.

Part I -- Bioreactor Engineering Specifications

- 1a. C1 Perfusion: Watson-Marlow 120U pump, 0.1-1.0 mL/min, silicone tubing (ID 1.6 mm), 100 mL reservoir Closed loop, medium change q3d
- 1b. C2 Compression: Bose ElectroForce 3200, 0-50 N, 0.01-10 Hz, sinusoidal waveform Porous platen (316L SS, 40% porosity)
- 2a. C3 RWV: Synthecon RCCS-4, 15-25 RPM, 50 mL vessel, silicone membrane oxygenation Horizontal rotation, scaffold free-floating
- 2b. C4 OoC: Custom PDMS, soft lithography, 400 um channels, 96-well format Gravity-driven or pneumatic perfusion
- 3a. C5 Integrated: C1 pump + C2 actuator + electrical stimulator (AM Systems 2100) + 6 sensors LabVIEW closed-loop control, 30 s cycle
- 3b. Sensors: PreSens O2 (C1,C5), Hamilton pH (C5), YSI glucose/lactate (C5), Honeywell force (C2,C5) All autoclavable, calibrated daily

Part II -- BPS Scoring Rubric

- 4a. Maturation: Tissue-specific metric / native tissue value, averaged across 4 tissues Capped at 1.0
- 4b. O2 Transport: Centre O2 tension / target O2 tension 5% target for cartilage, 20% for bone/cardiac/vascular
- 5a. Mechanical: $1.0 - |\text{measured strain} - \text{target strain}| / \text{target strain}$ Waveform fidelity assessed by FFT
- 5b. Scalability: Construct volume x parallel capacity, normalised to clinical need (10 cm³) OoC normalised separately for screening
- 6a. Monitoring: (Number of real-time parameters / 6) x (closed-loop = 1.0, open-loop = 0.7) 6 parameters = O2, pH, glucose, lactate, temp, force
- 6b. $\text{BPS} = 0.30 \times \text{Maturation} + 0.15 \times \text{O2} + 0.20 \times \text{Mechanical} + 0.15 \times \text{Scalability} + 0.20 \times \text{Monitoring}$