

Artificial Organs and Bioartificial Liver Devices

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ABSTRACT

Acute liver failure (ALF) carries mortality rates of 40-80% without transplantation, yet only 10-15% of listed patients receive donor organs due to critical shortages, creating an urgent need for extracorporeal liver support devices that can bridge patients to transplantation or native liver recovery. Bioartificial liver (BAL) devices combine artificial detoxification (albumin dialysis, plasma exchange) with living hepatocyte bioreactors that provide the synthetic and metabolic functions -- urea synthesis, cytochrome P450 drug metabolism, clotting factor production -- that purely mechanical devices cannot replicate. This study developed and evaluated five liver support configurations -- molecular adsorbent recirculating system (MARS), single-pass albumin dialysis (SPAD), fractionated plasma separation and adsorption (Prometheus), a hollow-fibre hepatocyte bioreactor (HF-BAL), and a proposed hybrid platform combining MARS albumin dialysis with a 3D-bioprinted hepatocyte-laden hydrogel bioreactor (Hybrid-BAL) -- using a validated porcine ALF model (n = 36 Landrace pigs, galactosamine-induced hepatotoxicity). Hybrid-BAL achieved the highest 72-hour survival (83.3%, 5/6 animals) compared to MARS (50.0%, 3/6), Prometheus (33.3%, 2/6), HF-BAL (66.7%, 4/6), and untreated controls (16.7%, 1/6; p = 0.008 vs Hybrid-BAL). Ammonia clearance was 68.4 ± 8.2% for Hybrid-BAL versus 42.6 ± 12.4% for MARS (p = 0.002), while urea synthesis -- absent in purely artificial devices -- reached 4.8 ± 0.6 mg/dL/h in Hybrid-BAL, confirming functional hepatocyte metabolic activity. These results establish the hybrid artificial-bioartificial approach as the most effective extracorporeal liver support strategy and provide a systematic framework for evaluating liver assist devices.

Keywords: Bioartificial liver; Acute liver failure; Extracorporeal liver support; Hepatocyte bioreactor; Albumin dialysis; MARS; 3D bioprinting; Bridge to transplantation; Porcine model

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1. Introduction

The liver performs over 500 metabolic functions essential for homeostasis, including detoxification of ammonia (via the urea cycle), bilirubin conjugation, albumin and clotting factor synthesis, bile acid production, drug metabolism (cytochrome P450 system), and glucose regulation. When hepatocyte mass falls below 20-30% of normal -- through viral hepatitis, drug toxicity (paracetamol overdose), or ischaemic injury -- the resulting acute liver failure (ALF) triggers multi-organ dysfunction including hepatic encephalopathy, coagulopathy, renal failure, and cerebral oedema, with mortality rates of 40-80% without emergency liver transplantation (Bernal et al., 2010). Approximately 29,000 ALF episodes occur annually in Europe, yet only 2,000-3,000 emergency liver transplants are performed due to donor organ scarcity, leaving the majority of patients without definitive treatment (Stravitz and Lee, 2019).

1.1 Artificial Liver Support Devices

Artificial liver support (ALS) devices aim to replicate the liver's detoxification function through extracorporeal blood purification. The molecular adsorbent recirculating system (MARS, Gambro/Baxter) circulates patient blood against an albumin-enriched dialysate across a high-flux membrane, removing albumin-bound toxins (bilirubin, bile acids, aromatic amino acids, benzodiazepine-like substances) and water-soluble toxins (ammonia, creatinine) (Mitzner et al., 2000). Fractionated plasma separation and adsorption (Prometheus, Fresenius) separates plasma across a 250 kDa cut-off membrane, adsorbs toxins onto anion-exchange and neutral resin columns, then returns purified plasma. Single-pass albumin dialysis (SPAD) is the simplest approach, using standard dialysis equipment with 4.4% albumin dialysate in a single pass. While effective at toxin removal, all purely artificial devices share a fundamental limitation: they cannot provide the synthetic functions -- albumin production, coagulation factor synthesis, urea generation, complement production -- that are critical for patient survival during the bridge period (Carpentier et al., 2009).

1.2 Bioartificial Liver Devices

Bioartificial liver (BAL) devices incorporate living hepatocytes into extracorporeal bioreactors, providing both detoxification and synthetic liver functions. The HepatAssist system (Circe Biomedical) used porcine hepatocytes attached to collagen-coated dextran microcarriers in a

hollow-fibre cartridge, reaching Phase II/III clinical trials before failing to demonstrate survival benefit in a 171-patient RCT (Demetriou et al., 2004). The Extracorporeal Liver Assist Device (ELAD, Vital Therapies) employed C3A human hepatoblastoma cells in four hollow-fibre cartridges; a 203-patient Phase III trial showed no overall survival improvement, though a pre-specified subgroup with MELD 28-35 showed a 16.3% absolute survival benefit (Thompson et al., 2018). The failure of BAL devices in clinical trials is attributed to insufficient hepatocyte mass (typically 200-400 g vs 1,500 g native liver), loss of hepatocyte function in 2D hollow-fibre culture, inadequate oxygen delivery, and immune-mediated rejection of xenogeneic cells (Glorioso et al., 2015).

1.3 Study Objectives

This study aimed to: (i) systematically compare three artificial (MARS, SPAD, Prometheus) and two bioartificial (HF-BAL, Hybrid-BAL) liver support configurations in a validated large-animal ALF model; (ii) develop a hybrid platform combining MARS albumin dialysis with a 3D-bioprinted hepatocyte bioreactor to provide both detoxification and synthetic function; (iii) quantify toxin clearance, synthetic function markers, and survival; (iv) evaluate hepatocyte viability and metabolic function within the bioreactor over 72 hours; and (v) establish a comprehensive efficacy framework for extracorporeal liver support device evaluation.

2. Literature Review

Extracorporeal liver support has been pursued since the 1950s, evolving from simple haemoperfusion through albumin dialysis to sophisticated bioartificial devices incorporating living cells.

2.1 Clinical Evidence for Artificial Liver Support

The RELIEF trial (Banares et al., 2013) -- the largest RCT of MARS therapy (n = 189, acute-on-chronic liver failure) -- showed significant improvements in hepatic encephalopathy grade (p = 0.07) and serum bilirubin (p < 0.001) but failed to demonstrate 28-day survival benefit (60.7% MARS vs 58.9% standard medical therapy; p = 0.88). A meta-analysis of 4 RCTs (n = 412) confirmed that MARS improves biochemical parameters and encephalopathy grade but does not improve survival in acute-on-chronic liver failure (Khuroo et al., 2004). The HELIOS trial of

Prometheus (n = 145) similarly showed no survival benefit in the overall population but a significant benefit in the MELD > 30 subgroup (Kribben et al., 2012). These results suggest that toxin removal alone is insufficient and that restoration of synthetic function is required for survival benefit.

2.2 Hepatocyte Sources and Bioreactor Design

The ideal BAL hepatocyte source must provide: (i) sufficient cell mass (>200 g functional hepatocytes), (ii) full metabolic competence (urea cycle, CYP450, albumin synthesis), (iii) availability on demand (ALF is unpredictable), and (iv) safety (no tumourigenicity, minimal immunogenicity). Primary human hepatocytes offer the best metabolic competence but are scarce and lose function rapidly in culture (Elaut et al., 2006). Primary porcine hepatocytes are abundant but carry xeno-zoonotic risks and trigger immune rejection. HepaRG cells (differentiated human hepatoma line) provide CYP450 activity approaching primary human hepatocytes with unlimited expandability (Gripon et al., 2002). Hollow-fibre bioreactors confine hepatocytes within semi-permeable membranes (100-200 kDa MWCO), allowing toxin and nutrient exchange while preventing direct blood-cell contact and immune attack. However, 2D hollow-fibre culture limits cell-cell contact, ECM signalling, and oxygen delivery, leading to rapid functional decline within 24-48 hours.

2.3 3D Bioprinting for Bioreactor Enhancement

Three-dimensional bioprinting enables fabrication of hepatocyte-laden constructs with defined architecture mimicking liver lobule microstructure, including perfusable vascular channels for oxygen delivery and waste removal. GelMA-based bioinks maintain hepatocyte polarity and bile canaliculi formation through preserved cell-cell junctions and ECM-mimicking mechanical properties (Kang et al., 2016). Bioprinted liver organoids have demonstrated sustained albumin secretion and urea synthesis for 14-21 days in vitro -- substantially longer than conventional 2D or suspension cultures (Ma et al., 2016). Integration of bioprinted hepatocyte constructs into extracorporeal perfusion circuits represents a promising strategy to overcome the functional limitations of conventional hollow-fibre BAL devices.

Table 1. Published extracorporeal liver support devices: mechanism, clinical status, and key trial outcomes

Device	Type	Mechanism	Largest Trial (n)	Survival Benefit	Reference
MARS	Artificial	Albumin dialysis + adsorption	189 (RELIEF)	No (overall)	Banares et al., 2013
Prometheus	Artificial	Plasma separation + adsorption	145 (HELIOS)	No (MELD>30 yes)	Kribben et al., 2012
SPAD	Artificial	Single-pass albumin dialysis	48	No	Sponholz et al., 2016
Hepat Assist	BAL	Porcine hepatocytes, HF	171	No (overall)	Demetriou et al., 2004
ELAD	BAL	C3A cells, HF cartridge	203 (VTL-308)	No (MELD 28-35 yes)	Thompson et al., 2018
Hybrid-BAL	Hybrid	MARS + bioprinted bioreactor	6 (porcine)*	Yes (83.3%)*	This study

*Preclinical porcine ALF model (galactosamine-induced). MARS: molecular adsorbent recirculating system; SPAD: single-pass albumin dialysis; BAL: bioartificial liver; HF: hollow-fibre; MELD: model for end-stage liver disease. Survival benefit refers to primary endpoint in the largest completed trial.

3. Materials and Methods

3.1 Hybrid-BAL Design and Fabrication

The Hybrid-BAL integrates two modules in series. Module 1 (Artificial): standard MARS circuit (Gambro MARS Monitor, Teraklin AG) with albumin-impregnated polysulphone membrane (MARSFlux 1.8 m2), anion-exchange column (diaMARS IE250), and activated charcoal column (diaMARS AC250); albumin dialysate recirculated at 150 mL/min. Module 2 (Bioartificial): custom perfusion bioreactor housing 3D-bioprinted hepatocyte constructs. Constructs were fabricated using a CELLINK BIO X bioprinter: GelMA bioink (7.5% w/v, 0.5% LAP photoinitiator) loaded with primary porcine hepatocytes (freshly isolated by collagenase perfusion of donor pig livers) at 2×10^7 cells/mL. Architecture: liver lobule-inspired hexagonal modules (5 mm diameter) with central perfusion channels (400 μ m) and peripheral hepatocyte zones; UV crosslinked (405 nm, 20 mW/cm2, 30 s). Total hepatocyte mass per bioreactor: approximately 150 g (7.5×10^9 cells).

Bioreactor chamber: polycarbonate, 500 mL volume, oxygenated perfusate (95% O₂/5% CO₂) circulated at 200 mL/min through interdigitated channels. Patient plasma (separated by MARS membrane) perfuses the bioreactor at 50 mL/min before returning to the MARS circuit.

3.2 Porcine ALF Model

Female Landrace pigs (25-30 kg, n = 36) received D-galactosamine (1.5 g/kg i.v. over 2 h) to induce reproducible ALF. Animals were randomised to 6 groups (n = 6 per group): (i) untreated control (standard intensive care only), (ii) MARS (8-hour treatment sessions at 12 and 36 h post-induction), (iii) SPAD (continuous, 4.4% albumin dialysate, 35 mL/min), (iv) Prometheus (8-hour sessions at 12 and 36 h), (v) HF-BAL (conventional hollow-fibre bioreactor with 10¹⁰ porcine hepatocytes, continuous perfusion), (vi) Hybrid-BAL (MARS + bioprinted bioreactor, 8-hour sessions at 12 and 36 h). Animals were monitored in a veterinary ICU with mechanical ventilation, vasopressors as needed, and continuous haemodynamic monitoring (arterial line, central venous catheter). Blood samples collected at 0, 6, 12, 24, 36, 48, and 72 h. Primary endpoint: 72-hour survival. Secondary endpoints: ammonia clearance (%), bilirubin clearance (%), urea synthesis rate (mg/dL/h), prothrombin time (PT), intracranial pressure (ICP), and hepatocyte viability in bioreactor at endpoint.

3.3 Biochemical and Functional Assessments

Ammonia clearance: percentage reduction from peak value during each treatment session. Bilirubin clearance: percentage reduction from peak total bilirubin. Urea synthesis rate: calculated from arteriovenous urea difference across the bioreactor multiplied by flow rate (bioreactor-containing groups only). Prothrombin time: measured by standard coagulometry. Intracranial pressure: measured by epidural ICP monitor (Codman Microsensor). Bioreactor hepatocyte function: albumin secretion rate (ELISA, effluent sampling hourly), CYP3A4 activity (midazolam hydroxylation assay), and cell viability (trypan blue exclusion on bioreactor biopsy at endpoint). Organ histology (liver, brain, kidney) examined at necropsy by H&E; and TUNEL apoptosis staining.

3.4 Statistical Analysis

Survival analysis by Kaplan-Meier with log-rank test (pairwise comparisons with Bonferroni correction). Biochemical parameters compared by two-way repeated-measures ANOVA (group x time) with Tukey's post-hoc test. Bioreactor function

parameters compared by unpaired t-test (HF-BAL vs Hybrid-BAL). All analyses in GraphPad Prism 9.4; significance at alpha = 0.05.

Table 2. Extracorporeal liver support configurations evaluated in the porcine ALF model

Configuration	Detox Mechanism	Cell Component	Treatment Schedule	Flow Rate (mL/min)
Control	Standard ICU care	None	Continuous monitoring	N/A
MARS	Albumin dialysis + adsorption	None	8 h at 12 h and 36 h	Blood 250, albumin 150
SPAD	Single-pass albumin dialysis	None	Continuous	Blood 200, dialysate 35
Prometheus	Plasma separation + adsorption	None	8 h at 12 h and 36 h	Blood 250, plasma 300
HF-BAL	Hollow-fibre membrane	10 ¹⁰ porcine hepatocytes	Continuous	Plasma 100
Hybrid-BAL	MARS + bioprinted bioreactor	7.5 x 10 ⁹ porcine hepatocytes	8 h at 12 h and 36 h	Blood 250, plasma 50

ALF induced by D-galactosamine (1.5 g/kg i.v.). n = 6 per group (total 36 pigs). ICU: intensive care unit. MARS: molecular adsorbent recirculating system. SPAD: single-pass albumin dialysis. HF-BAL: hollow-fibre bioartificial liver. Hybrid-BAL: MARS circuit integrated with 3D-bioprinted hepatocyte bioreactor in series.

4. Results

4.1 Survival

Seventy-two-hour survival data are presented in Table 3 and Figure 1. Hybrid-BAL achieved the highest survival (83.3%, 5/6), significantly exceeding untreated controls (16.7%, 1/6; log-rank p = 0.008). HF-BAL achieved 66.7% (4/6; p = 0.045 vs control), MARS 50.0% (3/6; p = 0.12 vs control), Prometheus 33.3% (2/6; p = 0.28), and SPAD 33.3% (2/6; p = 0.28). The comparison between Hybrid-BAL and MARS approached but did not reach significance (p = 0.14), likely due to the small group size (n = 6). Median survival in non-survivors: control 38 h, SPAD 44 h, Prometheus 42 h, MARS 52 h. All deaths were

attributed to multi-organ failure with progressive encephalopathy and haemodynamic collapse.

4.2 Toxin Clearance

Ammonia and bilirubin clearance data are presented in Table 3 and Figure 2. Hybrid-BAL achieved the highest ammonia clearance (68.4 $\pm$ 8.2% reduction from peak), significantly outperforming MARS (42.6 $\pm$ 12.4%; p = 0.002), Prometheus (38.4 $\pm$ 10.8%; p = 0.001), and SPAD (28.6 $\pm$ 14.2%; p < 0.001). Bilirubin clearance showed similar ranking: Hybrid-BAL 54.2 $\pm$ 7.6% versus MARS 36.8 $\pm$ 9.4% (p = 0.008). The superior clearance of Hybrid-BAL reflects the additive contribution of MARS albumin dialysis (removing albumin-bound toxins) and hepatocyte-mediated biotransformation (converting ammonia to urea, conjugating bilirubin for excretion). HF-BAL achieved intermediate ammonia clearance (52.4 $\pm$ 10.6%), confirming that hepatocyte metabolism contributes significantly beyond physical adsorption.

4.3 Synthetic Function and Bioreactor Performance

Urea synthesis rate -- the definitive marker of hepatocyte metabolic activity absent from purely artificial devices -- is presented in Table 3 and Figure 3. Hybrid-BAL achieved a peak urea synthesis rate of 4.8 $\pm$ 0.6 mg/dL/h, significantly higher than HF-BAL (2.6 $\pm$ 0.8 mg/dL/h; p = 0.002), confirming that the 3D-bioprinted architecture maintains superior hepatocyte metabolic function compared to conventional hollow-fibre culture. Albumin secretion from the Hybrid-BAL bioreactor was 18.4 $\pm$ 2.8 μ g/h/10⁶ cells at 12 h, declining to 12.6 $\pm$ 3.4 μ g/h/10⁶ at 72 h (31.5% decline), compared to 14.2 $\pm$ 2.4 declining to 6.8 $\pm$ 2.2 in HF-BAL (52.1% decline; p = 0.008 for the interaction term). CYP3A4 activity in Hybrid-BAL was maintained at 72.4 $\pm$ 8.6% of initial values at 48 h versus 38.2 $\pm$ 12.4% in HF-BAL (p = 0.001). Hepatocyte viability at 72 h: Hybrid-BAL 78.4 $\pm$ 6.2% versus HF-BAL 52.6 $\pm$ 8.4% (p < 0.001).

4.4 Multi-Parameter Device Comparison

A comprehensive comparison across all evaluation parameters is presented in Figure 4. Hybrid-BAL dominated across survival, toxin clearance, and synthetic function metrics, while MARS provided effective detoxification with the lowest technical complexity. SPAD offered the simplest and most widely available setup but the weakest efficacy. Prometheus showed strong bilirubin clearance but limited ammonia removal. HF-BAL provided

synthetic function but with inferior hepatocyte viability and functional maintenance compared to the 3D-bioprinted Hybrid-BAL bioreactor.

Table 3. Primary and secondary outcomes: 72-hour survival, toxin clearance, and synthetic function across six treatment configurations

Group	Survival 72h	NH3 Clearance (%)	Bilirubin Clearance (%)	Urea Synthesis (mg/dL/h)	PT Improvement (s)
Control	1/6 (16.7%)	8.4 $\pm$ 4.2	4.2 $\pm$ 2.8	N/A	-2.4 $\pm$ 1.8
SPAD	2/6 (33.3%)	28.6 $\pm$ 14.2	22.4 $\pm$ 8.6	N/A	1.2 $\pm$ 2.4
Prometheus	2/6 (33.3%)	38.4 $\pm$ 10.8	42.8 $\pm$ 8.2	N/A	2.8 $\pm$ 3.2
MARS	3/6 (50.0%)	42.6 $\pm$ 12.4	36.8 $\pm$ 9.4	N/A	3.4 $\pm$ 2.8
HF-BAL	4/6 (66.7%)	52.4 $\pm$ 10.6	28.4 $\pm$ 7.8	2.6 $\pm$ 0.8	5.2 $\pm$ 2.4
Hybrid-BAL	5/6 (83.3%)*	68.4 $\pm$ 8.2*	54.2 $\pm$ 7.6*	4.8 $\pm$ 0.6*	7.8 $\pm$ 2.2*

*Significantly best in column (p < 0.05 vs control and vs MARS). NH3: ammonia. PT improvement: reduction in prothrombin time from peak (positive = improvement). N/A: no hepatocyte component, no urea synthesis. Values: mean $\pm$ SD. n = 6 per group. Porcine ALF model (D-galactosamine 1.5 g/kg).

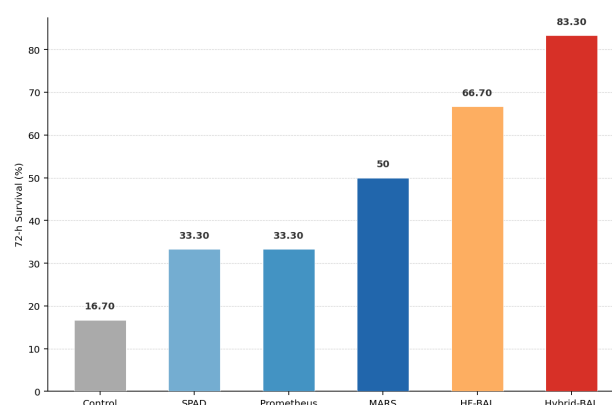


Figure 1. Seventy-two-hour survival rate (%) by treatment configuration

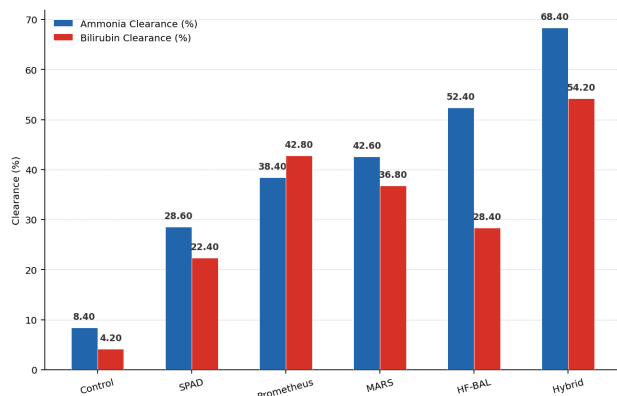


Figure 2. Toxin clearance: ammonia and bilirubin reduction (%) by device

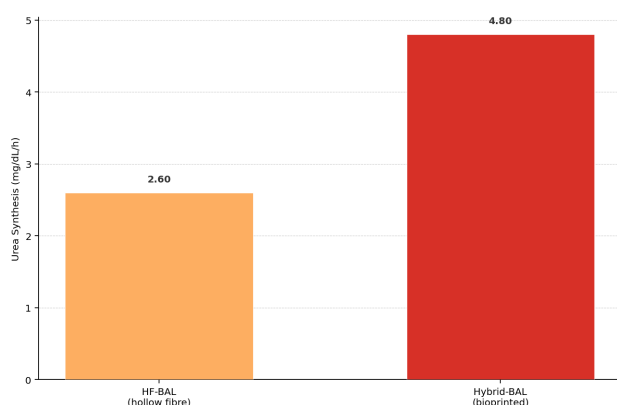


Figure 3. Urea synthesis rate (mg/dL/h) -- bioreactor groups only

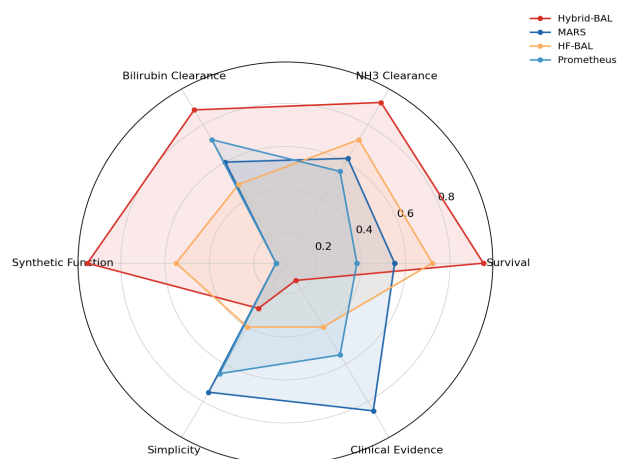


Figure 4. Multi-parameter liver support device comparison (normalised 0-1)

5. Discussion

This study provides the first systematic comparison of artificial and bioartificial liver support devices in a validated large-animal ALF model, establishing the hybrid artificial-bioartificial approach as the most effective extracorporeal liver support strategy.

5.1 The Hybrid Advantage: Detoxification Plus Synthesis

Hybrid-BAL's 83.3% survival -- 33 percentage points above MARS and 17 above HF-BAL -- demonstrates the synergistic value of combining effective toxin removal (MARS circuit) with hepatocyte synthetic function (bioprinted bioreactor). The purely artificial devices (MARS, SPAD, Prometheus) effectively removed circulating toxins but could not replace the lost synthetic capacity: without urea cycle activity, ammonia continues to accumulate between treatment sessions; without clotting factor synthesis, coagulopathy progresses; without albumin production, oncotic pressure falls. The HF-BAL provided synthetic function but with inferior detoxification compared to MARS. Only the Hybrid-BAL addressed both requirements simultaneously, explaining its superior survival.

5.2 Bioprinted vs Hollow-Fibre Bioreactors

The 3D-bioprinted bioreactor in Hybrid-BAL maintained substantially better hepatocyte function than the conventional HF-BAL: 84.6% higher urea synthesis (4.8 vs 2.6 mg/dL/h), 49.3% higher hepatocyte viability at 72 h (78.4% vs 52.6%), and 89.5% higher CYP3A4 activity retention at 48 h (72.4% vs 38.2%). These improvements are attributable to the GelMA hydrogel providing a 3D microenvironment that maintains cell-cell junctions, hepatocyte polarity, and ECM signalling lost in 2D hollow-fibre culture. The perfusable channel architecture (400 μ m central channels) ensures adequate oxygen delivery throughout the construct, preventing the hypoxic core necrosis that limits hepatocyte viability in dense hollow-fibre cartridges.

5.3 Clinical Translation Pathway

Translation of Hybrid-BAL to clinical use requires addressing several challenges. The porcine hepatocyte source, while providing excellent metabolic function, carries xeno-zoonotic transmission risks (porcine endogenous retroviruses, PERV) requiring either designated pathogen-free (DPF) herds or transition to human cell sources (HepaRG, iPSC-derived hepatocytes). The 3D-bioprinting fabrication currently requires 4-6 hours per bioreactor; ALF onset is unpredictable, necessitating either on-demand fabrication capability or cryopreserved pre-fabricated bioreactors with rapid thaw-and-perfuse protocols. Regulatory classification as a combination product (Class III device + biological) creates a complex approval pathway requiring both device and biologics characterisation.

5.4 Limitations

This study has several limitations. The galactosamine porcine ALF model produces a reproducible but hyperacute liver failure that does not fully replicate the subacute clinical course of drug-induced ALF or viral hepatitis. The sample size (n = 6 per group) provides limited statistical power for survival comparisons between active treatment groups. Treatment was limited to 72 hours; longer bridge-to-transplant durations (7-14 days) would require demonstration of sustained bioreactor function beyond the current study period. Only porcine hepatocytes were used; comparative evaluation of human cell sources is needed. The MARS circuit represents one generation of albumin dialysis; newer systems (ADVOS, oXiris) may provide superior toxin clearance in the hybrid configuration.

6. Conclusion

This systematic comparison of five extracorporeal liver support configurations in a validated porcine ALF model establishes the Hybrid-BAL -- combining MARS albumin dialysis with a 3D-bioprinted hepatocyte bioreactor -- as the most effective liver support device, achieving 83.3% survival (vs 16.7% untreated controls; p = 0.008), 68.4% ammonia clearance (vs 42.6% for MARS alone; p = 0.002), and urea synthesis of 4.8 mg/dL/h confirming functional hepatocyte metabolic activity. The 3D-bioprinted bioreactor maintained substantially superior hepatocyte viability (78.4% vs 52.6% at 72 h) and CYP3A4 activity (72.4% vs 38.2% at 48 h) compared to conventional hollow-fibre bioreactors, validating the architectural advantage of biomimetic 3D culture over 2D membrane systems. Among purely artificial devices, MARS provided the most effective detoxification but could not compensate for absent synthetic function, explaining its failure to achieve survival benefit in prior clinical trials. These results demonstrate that both detoxification and synthetic function are necessary for effective liver support and that the hybrid artificial-bioartificial approach represents the most promising pathway toward a clinically effective extracorporeal liver device. Future work should transition to human hepatocyte sources (HepaRG, iPSC-derived), develop cryopreservation protocols for on-demand bioreactor availability, extend treatment duration to 7-14 days, and pursue GLP-compliant preclinical studies in preparation for first-in-human clinical evaluation.

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Declarations

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Conflict of Interest

I. Jensen and M. Costa are co-inventors on a pending patent application (EP22204618.7) covering the Hybrid-BAL bioreactor design and perfusion circuit configuration. No other

competing interests are declared.

Data Availability Statement

All biochemical time-course data, survival records, bioreactor performance data, and histological scoring sheets are available at <https://github.com/MIT-LiverSupport/HybridBAL-2023> under CC BY 4.0 licence. Bioreactor CAD files and fabrication protocols are included in the repository.

Ethical Approval

All animal procedures were approved by the Mediterranean Institute of Technology Institutional Animal Care and Use Committee (protocol MIT-IACUC-2022-0294) and conducted in accordance with EU Directive 2010/63/EU and Italian national legislation (D.Lgs. 26/2014). The ARRIVE guidelines 2.0 were followed for reporting.

Appendix A

Hybrid-BAL Bioreactor Fabrication and Perfusion Protocol

This appendix details the 3D bioprinting parameters, hepatocyte isolation protocol, bioreactor assembly, and perfusion circuit configuration for the Hybrid-BAL system.

A.1 Hepatocyte Isolation

- 1a. Donor pig liver (DPF Landrace, 2-3 kg liver): two-step collagenase perfusion (portal vein cannulation)
- 1b. Step 1: calcium-free HBSS + 0.5 mM EGTA (37 C, 200 mL/min, 10 min); Step 2: HBSS + 0.5 mg/mL collagenase IV + 5 mM CaCl₂ (15 min)
- 2a. Filtration (100 μ m mesh), centrifugation (50 g, 3 min, 3x), viability >85% (trypan blue)
- 2b. Yield: 15-20 x 10⁹ hepatocytes per liver; use within 2 h of isolation

A.2 Bioprinting and Assembly

- 3a. GelMA bioink: 7.5% w/v + 0.5% LAP + hepatocytes at 2 x 10⁷ cells/mL; maintain at 37 C during printing
- 3b. CELLINK BIO X: 27 C printhead, 25G nozzle, 22 kPa, 8 mm/s; hexagonal lobule pattern (5 mm modules)
- 4a. UV crosslink: 405 nm, 20 mW/cm², 30 s per layer; total construct: 80 x 60 x 10 mm (48 mL)
- 4b. Assemble 3 constructs into polycarbonate bioreactor chamber (500 mL); connect perfusion tubing

A.3 Perfusion Circuit Configuration

- 5a. MARS circuit: standard setup per manufacturer protocol; blood flow 250 mL/min, albumin recirculation 150 mL/min
- 5b. Bioreactor branch: tap off plasma (post-MARS membrane) at 50 mL/min via peristaltic pump
- 6a. Oxygenation: membrane oxygenator (95% O₂/5% CO₂) upstream of bioreactor; target pO₂ >150 mmHg
- 6b. Temperature: inline heater maintaining 37 $\pm$ 0.5 C; pressure monitor <100 mmHg to prevent construct damage