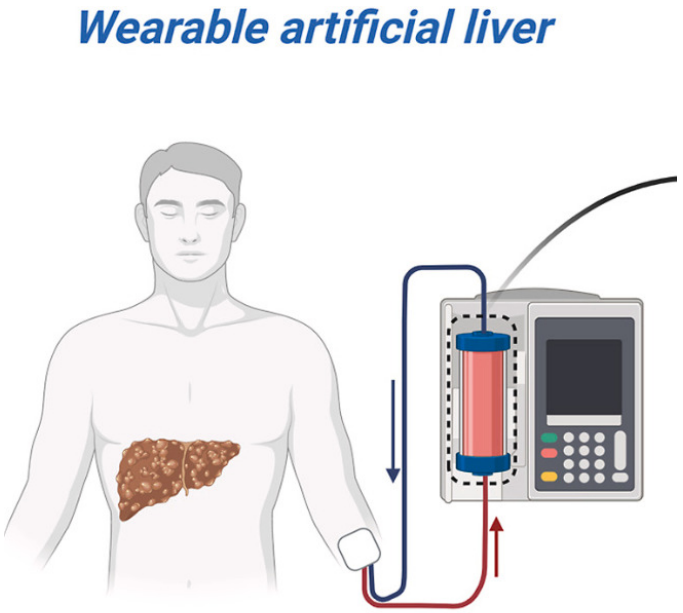
Topics Covered

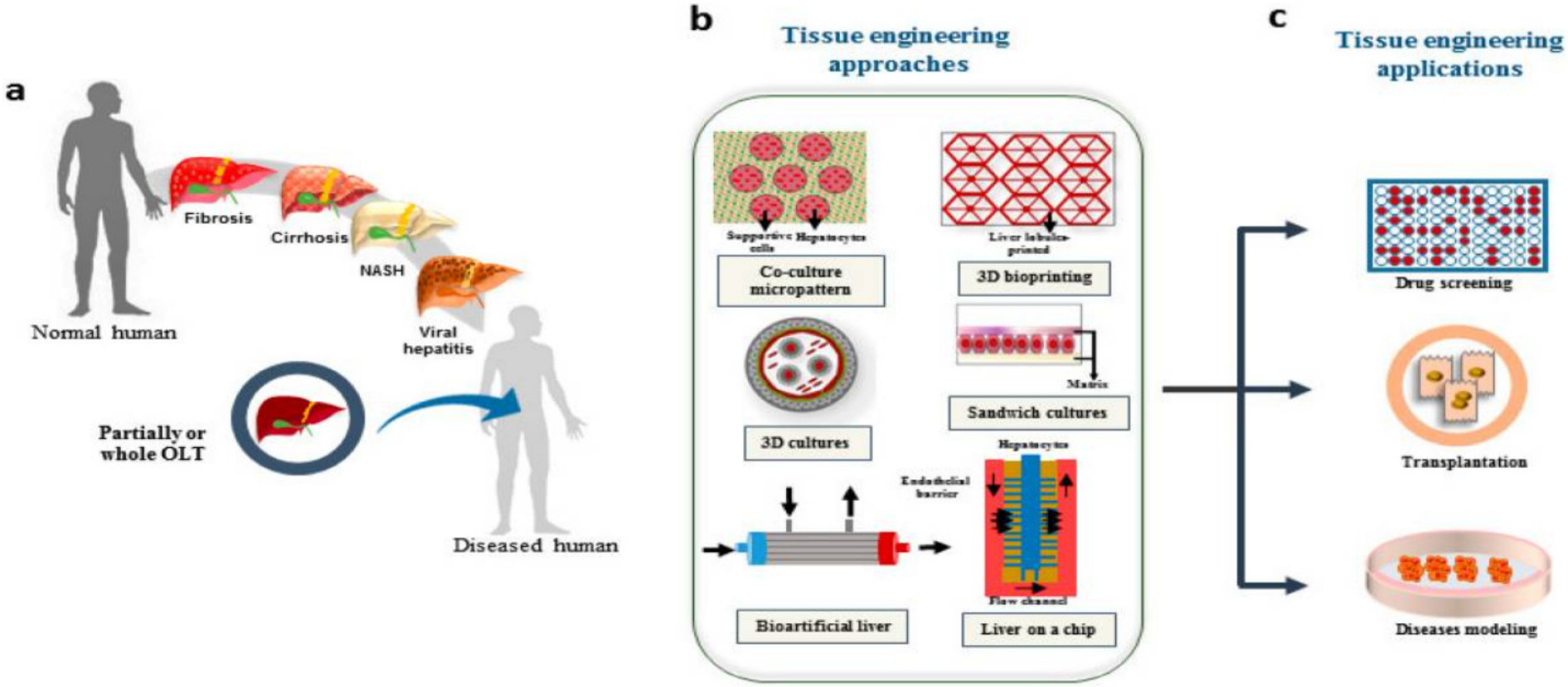
- Artificial Liver: Introduction, history and Significance
- Extracorporeal Liver Support Systems
- Artificial Liver Support Systems
- Types of Artificial Liver Support Systems
  - Hemoperfusion
  - Plasma Exchange
  - Molecular adsorbent recirculating system (MARS)
  - Prometheus
  - Single-Pass Albumin Dialysis (SPAD)
- Biological Artificial Liver Support Systems
- Types of Biological Artificial Liver Support Systems
  - Extracorporeal Liver Assist Device (ELAD)
  - Modular Extracorporeal Liver Support (MELS)
- Advantages and Disadvantages of different Liver Support Systems

Artificial Liver

- Liver tissue engineering (TE) enables us to reproduce and restore liver functions, fully or partially, which could be used in the treatment of acute or chronic liver disorders and/or generate an appropriate functional organ which can be transplanted or employed as an extracorporeal device. In this regard, a variety of techniques (e.g., fabrication technologies, cell-based technologies, microfluidic systems and, extracorporeal liver devices) could be applied in tissue engineering in liver regenerative medicine.
- Liver tissue engineering is an outstanding perspective of liver regeneration field, it builds an implantable or extracorporeal hepatoid organ by establishing a three-dimensional (3D) complex of cells and biomaterials
- Artificial liver systems are used to bridge between transplantation or to allow a patient's liver to recover. They are used in patients with acute liver failure (ALF) and acute-on-chronic liver failure.
- Bioartificial systems were developed to take over partially the synthetic and regulatory function of the liver besides detoxifying the patient's plasma. They use liver cells as biological component to accomplish this task. Different cell sources have been utilized in several systems.

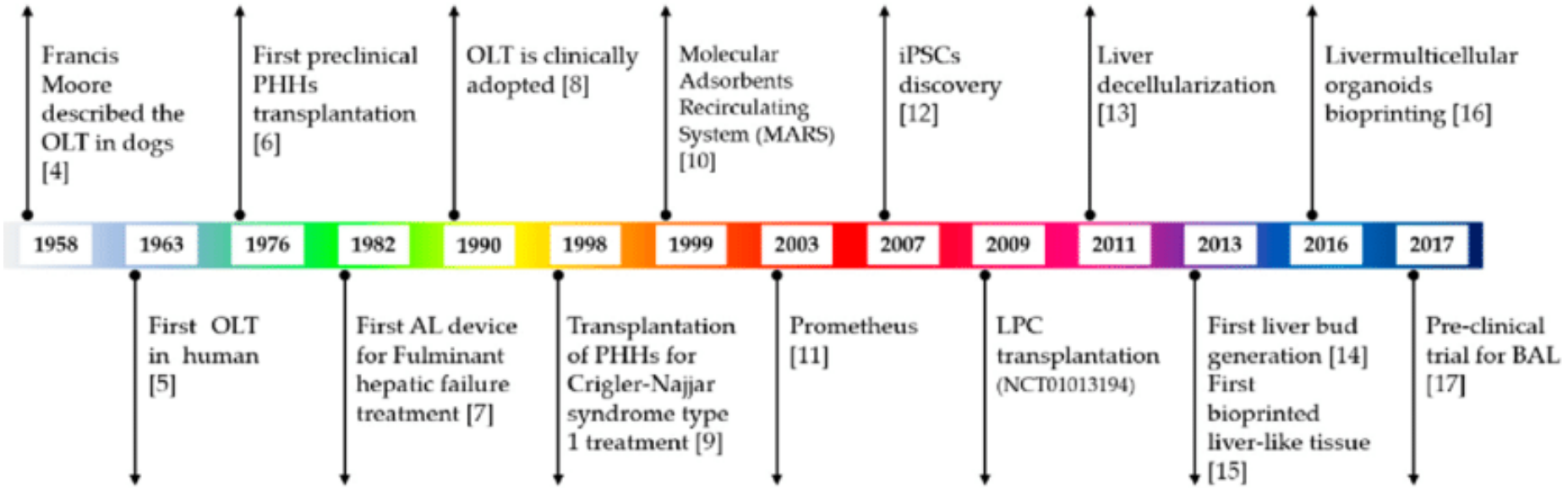


Artificial Liver



(**a**) Different diseases that result in liver failure; the only approved approach for end stage diseases is liver transplantation. (**b**) Different engineering approaches are growing to overcome the limitations in treatment of organ failure, drug screening, and disease modeling. (**c**) The possible applications which are promising using tissue engineering approaches. NASH: Nonalcoholic steatohepatitis; OLT: orthotopic liver transplantation.

Timeline



Schematic timeline of liver therapy and regeneration approaches and techniques [4-17]. OLT: orthotopic liver transplantation; PHHs: primary human hepatocytes; AL: artificial liver; iPSCs: induced pluripotent stem cells; LPC: liver progenitor cells; BAL: bio artificial liver.

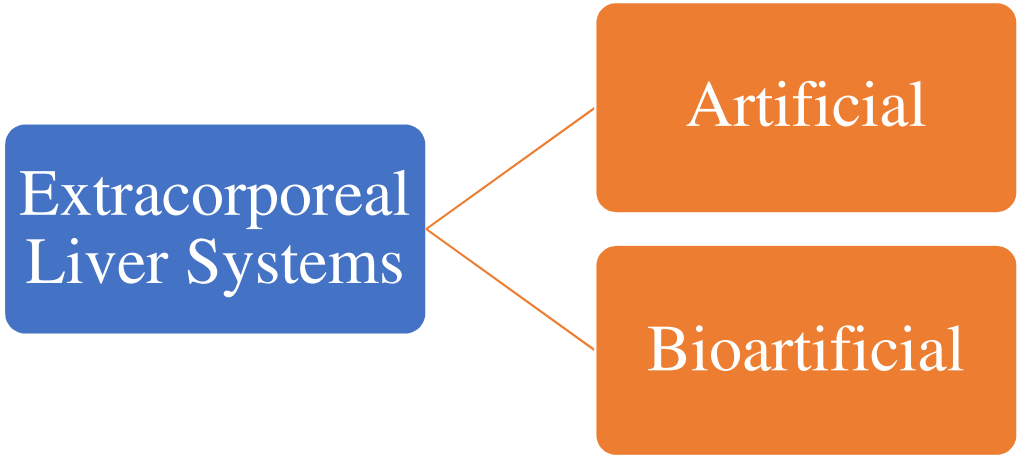
Necessity of Liver Tissue Engineering

- Liver diseases are associated with poor outcomes and are often considered a medical emergency due to the severity and complications associated with mortality. In 2016, liver diseases were responsible for more than one million deaths worldwide, and the trend has been clearly increasing in the last 10 years.
- Some of these deaths occur in the context of liver failure, in the form of either acute liver failure (ALF) or acute on chronic liver failure (AoCLF). In ALF, the adult mortality is approximately 50%, despite the increase in the number of patients receiving liver transplants.
- Regarding AoCLF, some recent studies show that one-third of patients hospitalized for cirrhosis with an acute complication develop AoCLF, and their mortality thus increases dramatically.
- In this context and given the shortage of organs for transplantation, efforts already have been developed to find therapeutic alternatives for patients who are waiting for a new organ (bridge-to-transplant) or who are not candidates for transplantation but for whom recovery is considered possible.
- Several systems based on the concept of albumin dialysis have been developed, the best-known being the following: the Molecular Adsorbent Recirculating System™ (MARS™), the Single-Pass Albumin Dialysis system (SPAD) and the Fractionated Plasma Separation and Adsorption system–FPSA (Prometheus™). However, these days Artificial devices or Extracorporeal devices are being utilized.
- It is generally assumed that a real effective artificial liver should be based on the capacity to perform the liver's multiple synthetic and metabolic functions, including detoxification and excretion.
- Hybrid bioartificial systems based on the presence of active functioning hepatocytes in an extracorporeal device that can be connected to the circulation of the patient with liver failure are promising.

Extracorporeal Liver Support Systems

- In the course of liver failure, water-soluble toxins (e.g., ammonia, mercaptans) and albumin-bound toxins (e.g., bilirubin, bile acids, aromatic amino acids, fatty acids) may accumulate and cause encephalopathy and dysfunction of other organs. While the field of detoxification and partially also of regulation can be addressed by artificial devices similar to dialysis (artificial systems, detoxification devices), the synthetic function of the liver can only be provided by living cells.

- Extracorporeal liver support systems aim to remove toxins (e.g., bilirubin, bile acids, aromatic amino acids, cytokines) accumulated in the circulation because of liver failure, mainly using an albumin dialysis–based technique.



Extracorporeal Liver Support Systems

Artificial	Biological
MARS	ELAD®
Fractionated plasma separation and adsorption (Prometheus)	BiologicDT
Single-pass albumin dialysis (SPAD).	Hepa-Mate™
Hemoperfusion	TEAK-BALSS/HBAL
Plasma exchange	AMC-BAL
Plasma adsorption	HEPATASSIST SYSTEM
Double plasma molecular absorption system (DPMAS)	

Classification of liver support systems

Artificial Liver Support Systems

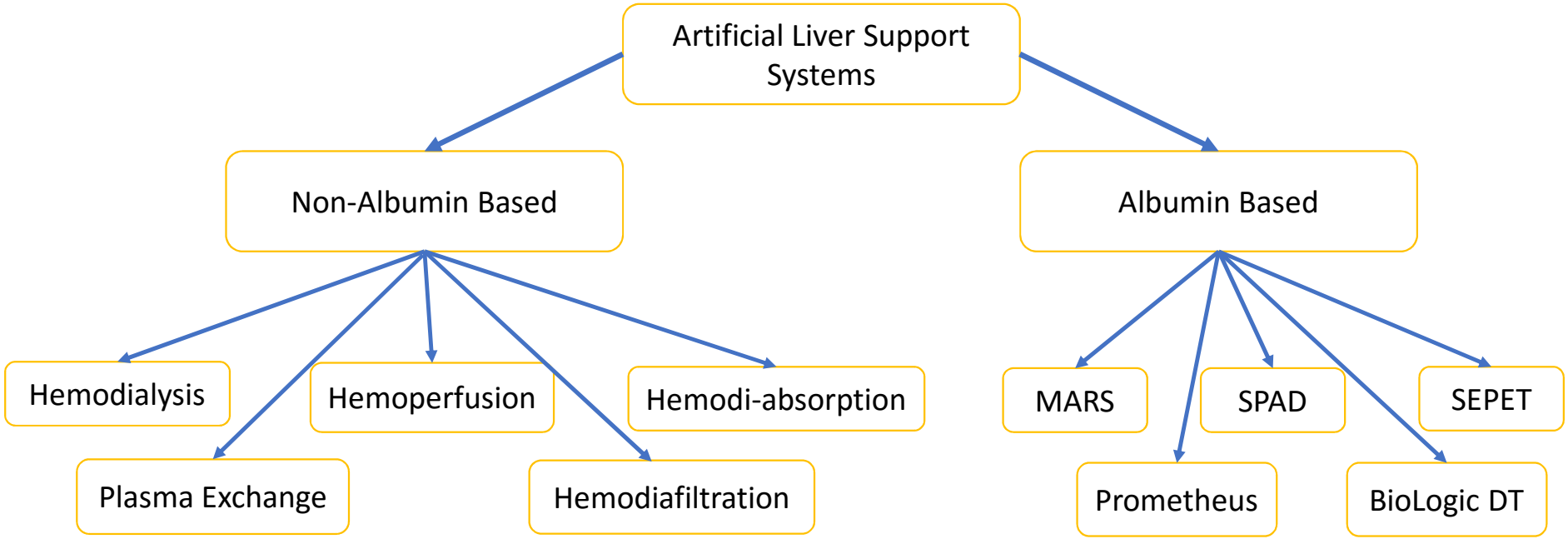
- Artificial liver devices (ALDs) usually work based on simple principles such as albumin dialysis, membrane filtration and the use of adsorbent columns to remove toxins. For the first time, in 1988, charcoal hemoperfusion helped patients with fulminant hepatic failure.

- Several Non-Biological devices have been used in clinical treatment, such as Liver Dialysis device, Molecular Adsorbent Recirculating System (MARS) and Prometheus device All kinds of Non-Biological treatments are based on toxin removal from blood.

- Advanced supporting systems such as Molecular Adsorbents Recirculating System, (MARS, Gambro, Sweden) developed by Stange et al. and Fractionated Plasma Separation, Adsorption and Dialysis system (FPAD, Prometheus, Fresenius Medical Care, Bad Hamburg Germany), have been clinically used to eliminate protein-bounded bilirubin and bile acids.

- MARS and SPAD use dialysis-based techniques in which, blood flow stream passes through a highly selective/small porosity (<50 kDa), high-flux membrane against an albumin-containing solution. In contrast, in plasma adsorption techniques, such as Prometheus system, non-selective membranes (approximately 250 kDa) are used and there is no parallel dialysate circuit

Types of Artificial Liver Support Systems



Artificial Liver Support Systems

(A) Non–Albumin-Based Devices	
Method	**Brief Explanation**
Hemodialysis	In 1958 Kiley et al. described the symptomatic and clinical improvement in form of improved neurological status in four of the five patients of ammonia intoxication treated by hemodialysis. However, no benefit was noted in long-term survival of these patients.
Charcoal hemoperfusion	Initially used in the treatment of barbiturate poisoning, charcoal hemoperfusion has been shown to remove many water-soluble molecules associated with encephalopathy in hepatic failure patients.
Hemodi-absorption	This is a procedure that has the capability of removing toxins of less than 5 kDa. These include aromatic amino acids, glutamine, mercaptans, benzodiazepine-like substances, false neural transmitters, ammonia, and manganese.
Plasma exchange TPE (Therapeutic Plasma Exchange) HVP (High Volume Plasma exchange)	Plasma element is separated from cellular blood components of blood by using a hollow fiber filter made of cellulose diacetate and polyethylene membrane or other synthetic materials.
Hemodiafiltration	This is a combination of hemodialysis and hemofiltration. Hemodialysis is useful for removing molecules which are less than 5 kDa and hemofiltration can remove molecules in the 5–10 kDa range. A high-performance membrane such as a large-pore sized poly methyl methacrylate (PMMA) membrane is performed.

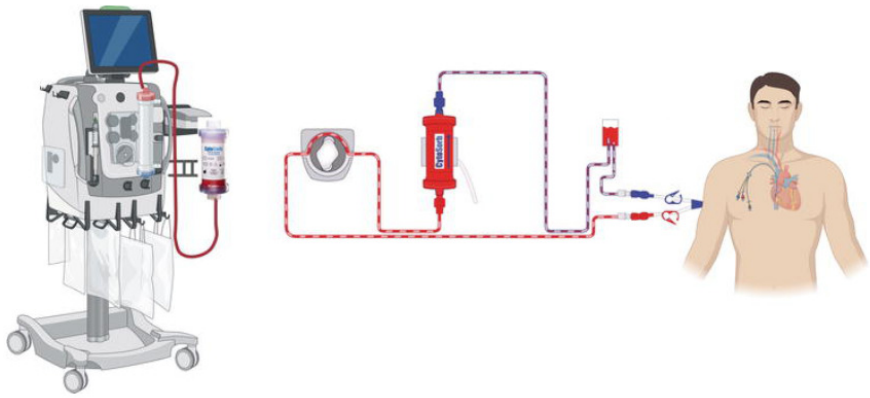
Artificial Liver Support Systems

(B) Albumin-based systems

Company	Brief explanation
MARS® (molecular adsorbent recirculating system)	Uses a high-flux hollow-fiber hemodiafilter and albumin as the acceptor molecule for albumin-bound toxins within the extracorporeal circuit
Prometheus	Based on an albumin-permeable polysulfone membrane, which enables the patient's albumin fraction to pass into a secondary circuit in which the direct purification from albumin-bound toxins by different absorbers (that is, anion exchanger and neutral resin) takes place.
SPAD (single-pass albumin dialysis)	It uses a standard continuous renal replacement therapy system without any additional columns or circuits. Blood is dialyzed against a standard dialysis solution with the addition of 4.4% albumin in the dialysate.
SEPET (selective plasma filtration therapy)	Combines aspects of fractionated plasma separation, adsorption and single-pass albumin dialysis. The fractionated plasma passes through an albumin-permeable size-selective membrane.
BioLogic-DT (later Liver Dialysis System™ [HemoCleanse, Lafayette, IN, USA])	Based on a cellulosic plate dialyzer with a suspension of powdered charcoal and cation exchangers as dialysates, is no longer marketed.

Hemoperfusion

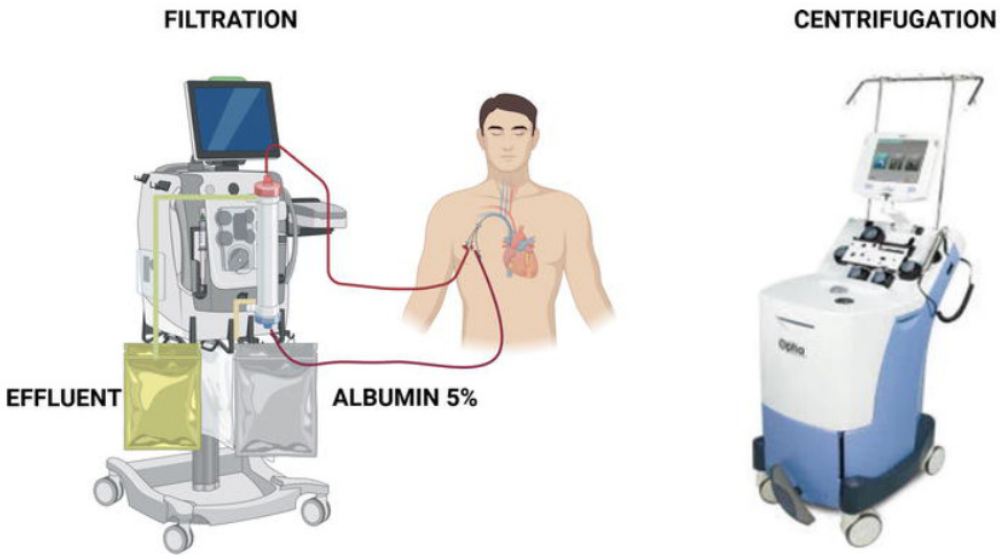
- There are many reports of the use of hemoperfusion in liver failure since the 70s and 80s.
- Hemoperfusion is an extracorporeal therapy technique, which allows the passage of blood through a filter with the adsorption capacity of molecules with molecular weights from 5 to 50 kD, and the cartridges are classified according to a) composition in natural compounds (carbons) and synthetics (divinylbenzene), b) surface and volume, c) size, and d) selectivity.
- The adsorption mechanisms attract solutes through different forces (hydrophobic interactions, ionic attraction, hydrogen bonding, and Van der Waals interactions), which allow the uptake of PAPMs, DAMPs, cytokines, chemokines, and multiple toxic substances (drugs, poisons).



Hemoperfusion. It is an adsorptive therapy that uses activated carbon cartridges or divinylbenzene resins.

Plasma exchange

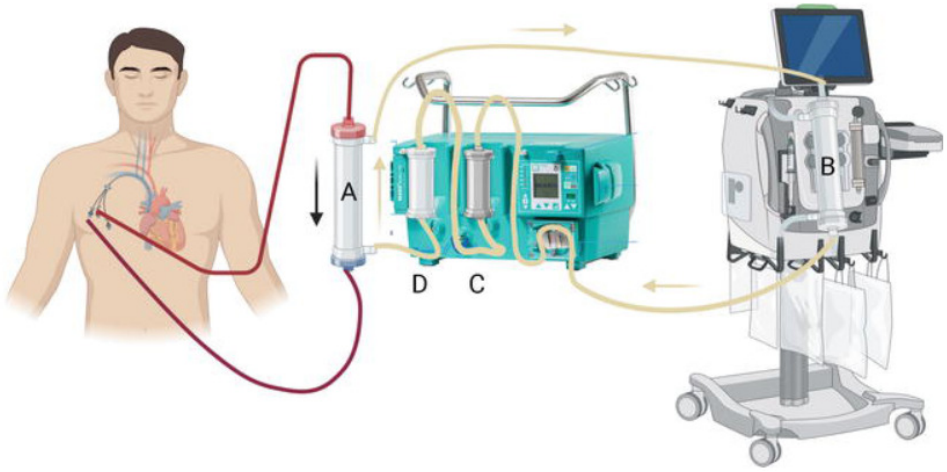
- The plasma exchange (PE) is an extracorporeal purification technique that is carried out by centrifugation or filtration.
- This last technique uses a high permeability membrane with a large pore size greater than 0.3 microns, allowing the separation of plasma and the removal of medium and medium molecules with high molecular weight, such as cytokines and immunoglobulins.



Plasma exchange (PE). The technique uses a high permeability membrane with a large pore size that allows plasma separation and replacement of the extracted volume is performed with 5% albumin or fresh frozen plasma.

Molecular adsorbent recirculating system (MARS)

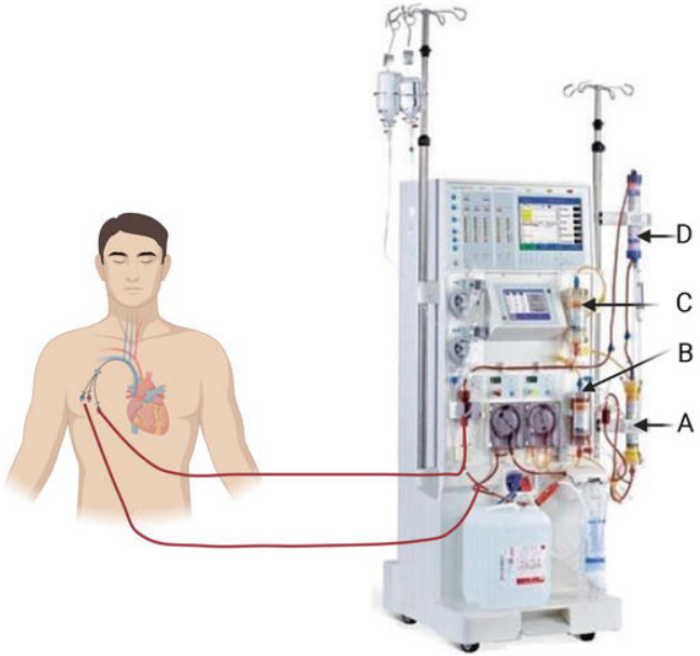
- This purification system allows the elimination of toxins bound to albumin and water-soluble toxins.
- The blood that is extracted by catheter circulates at a blood flow of 200 ml/min and is placed in contact with the high permeability MARS® FLUX 2.1 filter (60 kD) and 600 ml of dialysate albumin circulates counter currently with pumped 150 ml/min.
- This recirculated albumin is placed in contact with the diaFLUX 1.8 filter and with the conventional dialysis bath of the Prismaflex System, allowing the elimination of water-soluble molecules, and the regenerated albumin circulates through the activated carbon cartridge (diaMARS® AC250) that captures cationic toxins and then goes to a second resin cartridge (diaMARS® IE250) that captures anionic toxins.
- The procedure is performed with an average of 8 hours up to date, with the aim of reducing total bilirubin by more than 25% in each session, achieving a reduction in nitrogen and ammonia and reversing encephalopathy.



Molecular adsorbent recirculating system (MARS). A) Once the patient's blood enters the high permeability (60 kD) MARS® FLUX 2.1 filter, 600 ml of dialysate albumin circulates in a countercurrent direction with pumped 150 ml/min. B) Then, the recirculated albumin is directed to the diaFLUX 1.8 filter and with the conventional dialysis bath of the Prismaflex system, allowing the elimination of water-soluble molecules. C) Next, the regenerated albumin circulates through the activated carbon cartridge (diaMARS® AC250) that captures cationic toxins. D) It is then directed to a second resin cartridge (diaMARS® IE250).

Prometheus

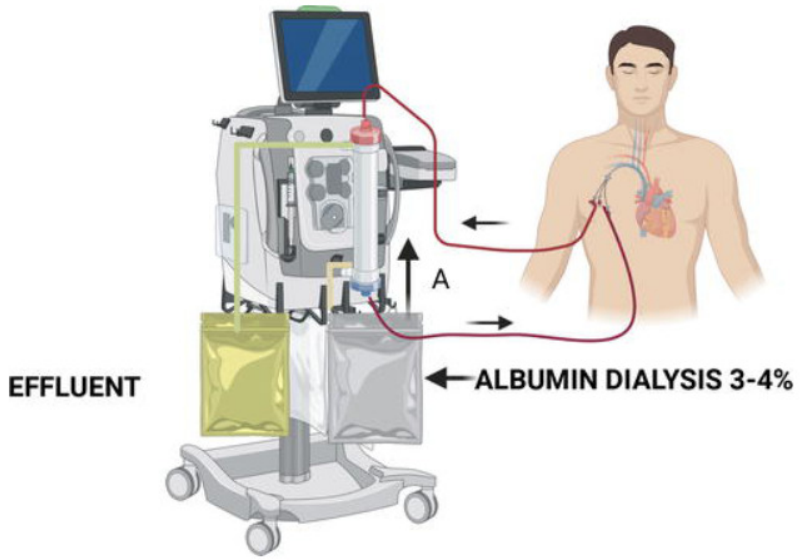
- It is a fractionated plasma separation and adsorption system, the blood extracted through a catheter circulates through an AlbuFlow® AF01 filter, with a high screening coefficient (250 kD), which separates the albumin from the blood.
- The first to pass through the plasma to an adsorbent cartridge Prometh® 01 contains a neutrally charged, highly porous resin that absorbs bile acids, aromatic amino acids, and phenols.
- Then, the plasma and the albumin circulate through a second Prometh® 02 cartridge, which is an anion exchange resin in the form of chloride that allows the absorption of bilirubin, and following the sequence of the circuit, the blood plasma and the detoxified albumin are returned to the Fresenius® helixone high-flow filter to remove water-soluble toxins.



Fractionated plasma separation and adsorption (Prometheus). A) The blood extracted through a catheter circulates through an AlbuFlow ® AF01 filter. B) Albumin and separated plasma pass to a Prometh® 01 adsorbent cartridge. C) Albumin then circulates through a second Prometh® 02 cartridge. D) Following the circuit sequence, blood plasma and detoxified albumin are returned to the Fresenius® helixone high-flow filter.

Single-pass albumin dialysis (SPAD)

- This purification system that uses the physical foundation of diffusion with a dialysis bath enriched with albumin in 3–4% concentrations that act as a binder for substances bound to proteins.
- This technique uses a continuous renal replacement therapy (CRRT) machine in continuous venovenous hemodialysis modality, with a dialysate flow of 700–1000 ml/min.
- The dialysate flow with albumin will allow the capture of lipophilic molecules present in the patient's blood that will bind to the albumin that circulates in the countercurrent direction to the blood flow through the filter, allowing the elimination of bilirubin, bile acids, and nitrogen acids.
- This technique does not use additional cartridges or other extracorporeal circulation machines and can be performed in low-income countries.
- There is evidence that SPAD allows a significant decrease in bilirubin similar to MARS, but it failed to lower bile acid and cytokine values

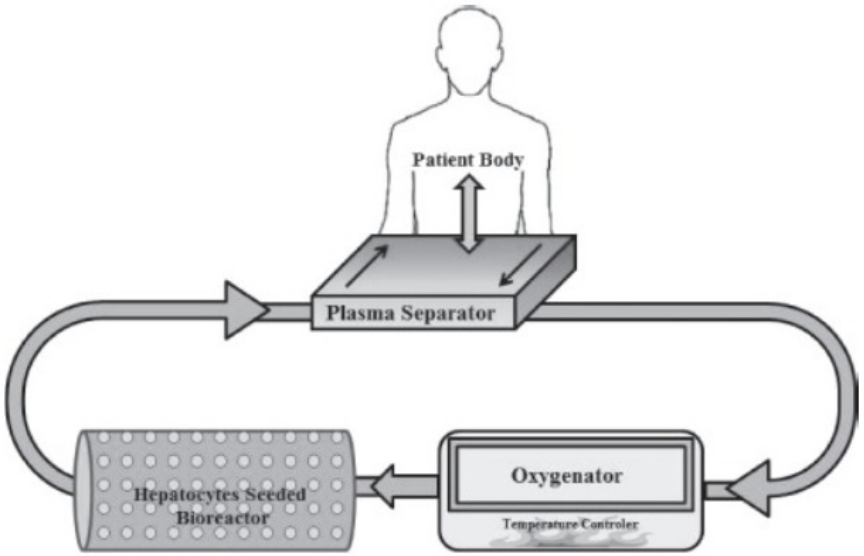


Single-pass albumin dialysis (SPAD). A) The technique uses a continuous renal replacement therapy (CRRT) machine in continuous venovenous hemodialysis modality, with a dialysate flow of 700–1000 ml/min, the dialysate is enriched with 3–4% albumin.

Biological Artificial Liver Support Systems

- All kinds of Non-Biological treatments are based on toxin removal from blood. However, liver function is so complex that it can not be substituted by the detoxification functions of NBL alone. It is theoretically possible that Biological Artificial devices containing a cell-filled bioreactor could replace most important functions of liver.
- Bioartificial systems (BALs) were developed to take over partially the synthetic and regulatory function of the liver besides detoxifying the patient's plasma. They use liver cells as biological component to accomplish this task.
- BAL devices require a minimal number of 10^{10} functional hepatocytes. This number represents almost 10% of total liver mass in adults.
- Different cell sources that have been used in Biological Artificial devices include: immortalized human hepatocyte cell lines, primary porcine and human hepatocytes.
- Based on device configuration, different types of BALs are available. Hollow fiber devices, packed beds, flat plate systems and encapsulation-based reactors are common examples of BALs.
- To the best of our knowledge, BAL devices which are currently under clinical investigation include HepatAssist device, Extracorporeal Liver Assist Device (ELAD), Bioartificial Liver Support System (BLSS), Academic Medical Center-Bioartificial Liver (AMC-BAL) and Modular Extracorporeal Liver Support device (MELS)

Biological Artificial Liver Support Systems



A proposed set up of bioartificial liver (BAL) system. The system comprises the filtration of patient venous blood passes through plasma separator unit connected to oxygenator unit under a controlled temperature. Furthermore, the plasma is then passed through hepatocytes activated bioreactor and return back to the patient body along with the blood cells.

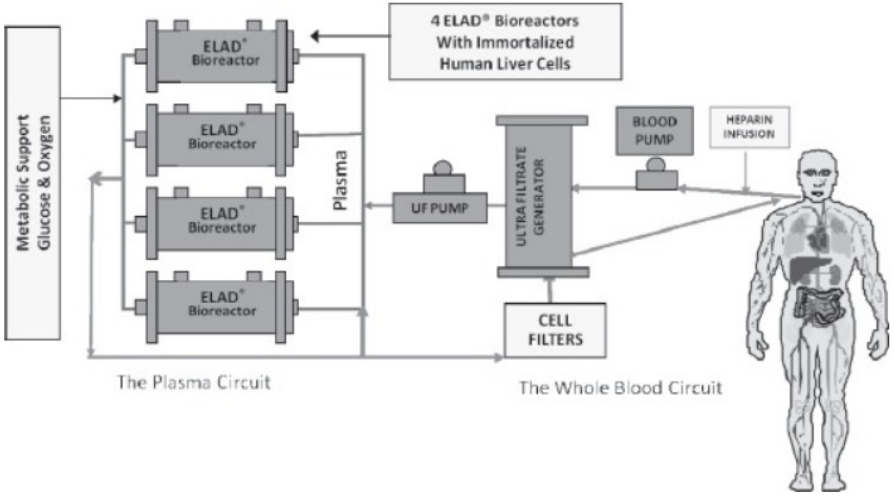
Biological Artificial Liver Support Systems

Commercially available bio-artificial liver devices (BAL)

Bio-artificial Liver Systems		
Company	**Bioactive Functional Cells**	**Explanation**
HepatAssist	Cryopreserved Porcine hepatocytes (7 × 10^9 cells)	Plasma is separated from blood cells and then the plasma is circulated through the bioreactor after first passing through a charcoal filter and an oxygenator.
ELAD® (Extracorporeal Liver Assist Device)	Hepatoblastoma cell line HepG2-C3A (200–400 g)	The cells are isolated from the patient's plasma by hollow-fiber membranes. An integrated charcoal absorber, and a membrane oxygenator supports detoxification and maintains the oxygen supply of the cells.
AMC-BAL (Amsterdam Medical Center-Bioartifcial Liver device)	Porcine hepatocytes (10–14 × 10^9 cells)	The plasma is in direct contact with the cells, lead to better mass exchange between cells and the patient's plasma.
MELS (Modular Extracorporeal Liver Support)	Human hepatocytes (up to 650 g)	The bioreactor is composed of a three-dimensional matrix interwoven with bundles of hollow fibers. The hollow fibers have a molecular cutoff weight of 400 kDa and used to perfuse patient's plasma adjacent to the functional hepatocytes.
BLSS (Bioartificial Liver Support System)	Porcine hepatocytes (70–120 g)	Whole blood, rather than plasma, is passed through the fibers after warming and oxygenation.

Extracorporeal Liver Assist Device

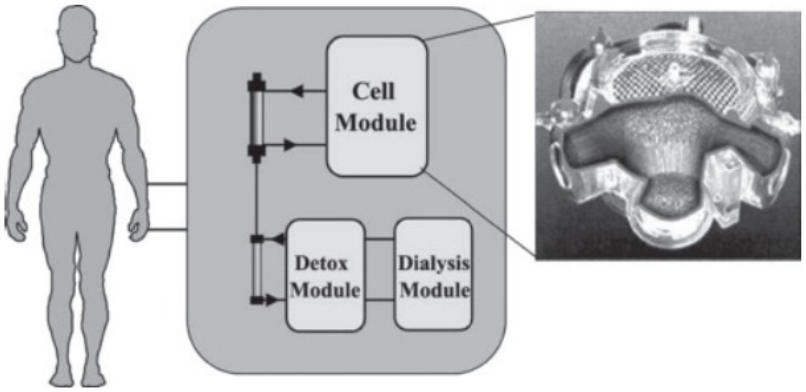
- ELAD (Vitagen Inc., formerly Hepatix Inc., La Jolla, CA) has created the first medical device to incorporate "immortalized" human liver cells, i.e., "C3A" cell line.
- This cell line is a highly differentiated clonal population isolated from a human hepatoblastoma cell line (HepG2). Cells are originally seeded and grown in the extracapillary space of a hemodialysis cartridge containing approximately 10,000 cellulose acetate-based hollow fibers.
- The device is then perfused with the patient's plasma through the cartridge containing hepatocytes.
- A hemodialysis-type catheter carries blood to an ultrafiltration device with the ultrafiltrate (plasma) flowing through the ELAD being exposed to the metabolic activities of the immortalized liver cells, which execute some activities like gluconeogenesis, ureagenesis, and P450 activity but not all of the normal liver's metabolic function.
- VitaGen C3A cells in the ELAD cartridge produce several human liver-specific proteins, metabolize drugs, use galactose, and reproduce very well in culture. Finally, the treated plasma is then filtered and returned back to the patient body



Schematic illustration of extracorporeal liver assist device (ELAD) designed by Vitagen Inc. ELAD uses immortalized human liver cells seeded cellulose acetate based hollow fiber bioreactor. [*Figure courtesy of Vital Therapies Inc. TM, CA*].

Modular Extracorporeal Liver Support

- MELS is designed by combining the different extra-corporeal medical devices together.
- The cell module is composed of a bioreactor loaded with primary human liver cells isolated from discarded donor livers, which are unsuitable for transplantation.
- In this device, an additional dialysis module performing continuous venovenous hemodiafiltration is also used.
- The bioreactor consists of hollow fiber membranes and hydrophobic membranes interwoven to four independent compartments. Of these four compartments, two allow perfusion of the liver cells with the patient's plasma and one is used for integrated oxygenation as well as carbon dioxide elimination.
- The fourth compartment used for the seeding of liver cells between the hollow fibers.
- In between the void space of fibers, cells grow, adhere, and form active cell aggregates. This leads to the hollow fibers getting interwoven into a three-dimensional capillary network with numerous subunits enabling exchange of decentralized mass with an adequate amount of nutrient supply and effective metabolite removal



Schematic representation of Modular Extracorporeal Liver Support (MELS). The MELS consists of a multi-compartment hollow fiber bioreactor (CellModule), a toxin removal albumin dialyzer (DetoxModule) and a kidney dialyzer (DialysisModule). The system uses multi-interwoven independent compartments, where cells are present in separate compartment with hollow fibers. [*Figure reproduced with permission from Kobayashi et al. J Artif Organs 2003;6:236–44*].

Limitations of Extracorporeal Liver Systems

Table 1. Advantages and Disadvantages of Each Liver Support-System Method.

No.	Liver Support Method	Advantages	Disadvantages
1.	Single-Pass Albumin Dialysis (SPAD)	Significant reduction (>50%) in total plasma bilirubin, conjugated bilirubin, blood urea nitrogen, and creatinine	Clinical studies about effectiveness in toxin removal are still limited (only 1–2 studies from the last five years).
3.	Molecular Adsorbent Recirculation System (MARS)	Eliminate toxins, and repair liver failure symptoms (toxin blood level 0%) Reduction in bilirubin, bile acid, ammonia, urea, lactate, and creatinine levels (>50% compared to previous levels) MARS can stabilize the anhepatic condition in ALF and ACLF for 96 h before transplantation. Effective in clearing bile acid compared to SPAD (bile acid clear 100%) A significant increase (50%) in mean arterial pressure and systemic blood circulation resistance without a change in heart index. Plasma renin activity reduction (up to 50%).	The survival rate has not been reported (no studies). The mortality percentage did not significantly reduce (compared to the previous study, 0% reduction). Thrombocytopenia, coagulopathy, and bleeding risks up to 20–30%. MARS is relatively more expensive (cost >1000 USD in Europe)
4.	Bio-artificial Liver Support System (BALSS)	Improve neurological state and liver and kidney functions to bridge transplantation (the function status is usually based on assessment score).	BALSS might not reduce ALF deaths, but the survival rate is still controversial. It can either reduce or increase ALF deaths (the percentages are 50%) The delivery of bioreactors is expensive and impractical (it costs USD >500) Limited study and case reports (there have only been 1–2 studies in the last five years.) The risk of zoonosis disease transmission is up to 30%.