LECTURES 10-11: ARTIFICIAL LIVER

10.1 Introduction

The liver plays a major role in metabolism and has several functions in the body including glycogen storage, plasma protein synthesis, and drug detoxification. This organ is the largest gland in the human body. It produces bile, which is important in digestion. It performs and regulates a wide variety of high-volume biochemical reactions requiring specialized tissues. Medical terms related to the liver often start with hepato- or hepatic from the Greek word for liver, *hepar*.

The liver is among the few internal human organs capable of natural regeneration of damaged or lost tissue; as little as 25% of the remaining liver can regenerate into a whole liver. This is predominantly due to the hepatocytes acting as potential stem cells.

10.2 Artificial Liver

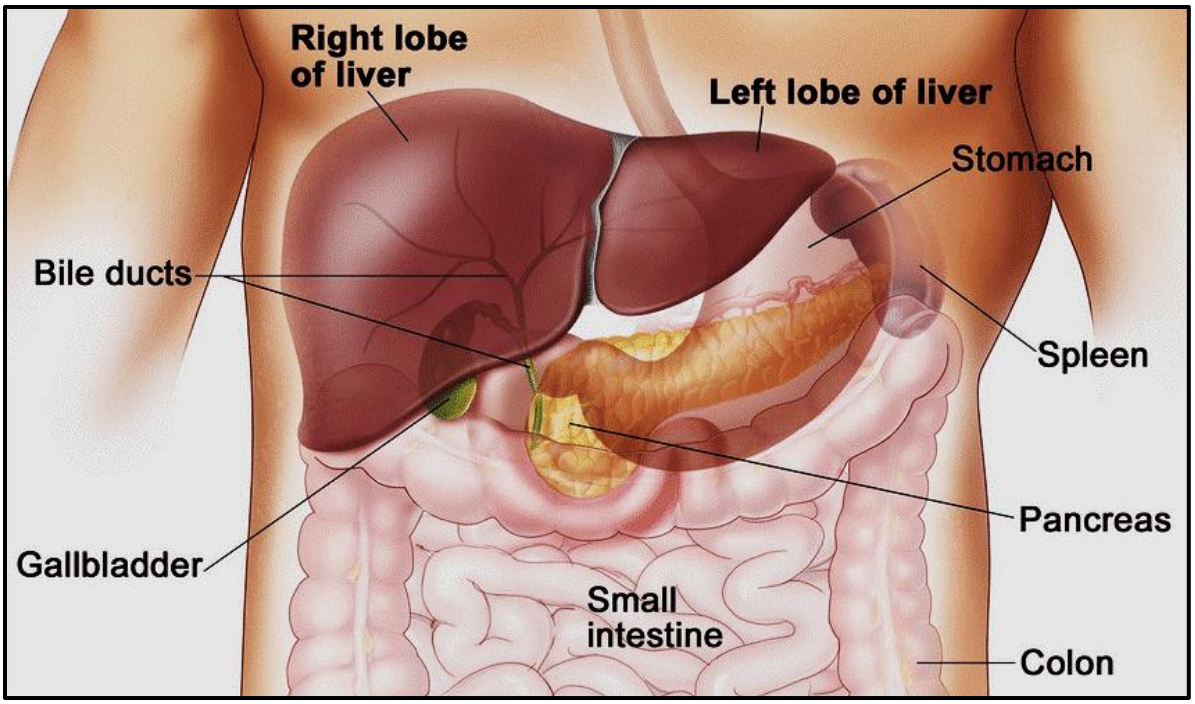
Enthusiasm for liver support devices, particularly cell-based biological systems and albumin dialysis, has increased over the last decade resulting in considerable clinical activity both within and without the construct of clinical trials. Most data have been generated on patients with acute liver failure or patients with decompensation of chronic liver disease, often referred to as acute-on-chronic liver failure.

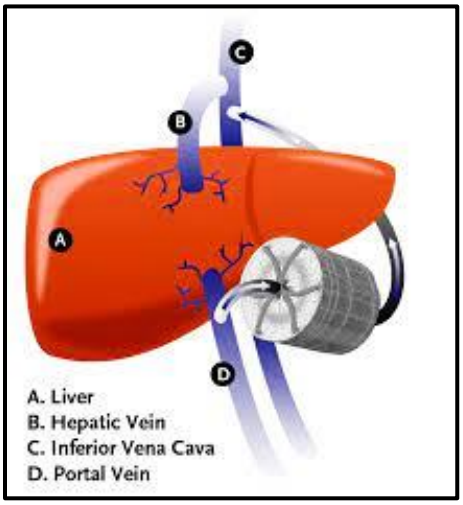
In clinical use for acute liver failure, bridging to liver transplantation is a more realistic goal rather than transplant-free survival. In actinophonic liver failure, the objective of attaining clinical stability with treatment appears more achievable.

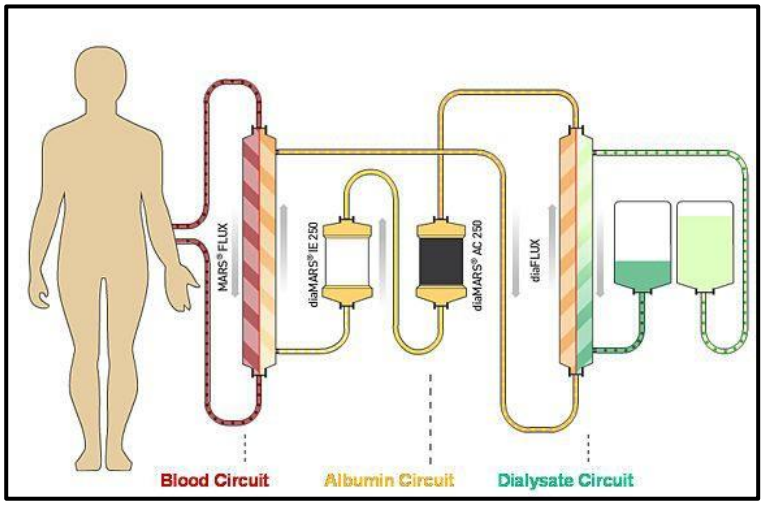
Currently, there is no single artificial organ or device capable of emulating all the functions of the liver. Some functions related to the removal of toxic substances can be emulated by liver dialysis, charcoal hemoperfusion or plasma exchange, experimental treatments for liver failure.

These methods have not yet been shown to improve the survival of patients with liver failure, although hemodialysis did work well on renal failure associated with liver failure. A small clinical trial (n=5) using a slow plasma exchange in combination with high-flow continuous hemodiafiltration showed some promise. The most promising medical device approaches at this moment are SPAD (single pass albumin dialysis) and MARS (molecular adsorbent recycling system), which combines conventional dialysis with albumin dialysis.

Medical device-based artificial liver support systems have a beneficial influence on the neurological state of patients but do not improve survival. More beneficial effects have been expected from systems that bring the blood of the patient in contact with living liver cells, or: bioartificial liver systems (BAL). The cell activity can then contribute to the compensation of the failing patient's liver, by e.g. detoxification, biosynthesis, and biotransformation. The BAL may be developed as a bridge to transplant for patients suffering from acute-on-chronic liver failure or for some patients as a bridge to recovery.







10.3 The liver functions:

- The liver produces and excretes bile required for emulsifying fats. Some of the bile drains directly into the duodenum, and some is stored in the gallbladder.
- The liver performs several roles in carbohydrate metabolism:
  - Gluconeogenesis; the synthesis of glucose from certain amino acids, lactate or glycerol
  - Glycogenolysis; the breakdown of glycogen into glucose
  - Glycogenesis; the formation of glycogen from glucose
  - The breakdown of insulin and other hormones
- The liver is responsible for the mainstay of protein metabolism.
- The liver also performs several roles in lipid metabolism:
  - Cholesterol synthesis
  - The production of triglycerides (fats).
- The liver produces coagulation factors I (fibrinogen), II (prothrombin), V, VII, IX, X and XI, as well as protein C, protein S and antithrombin.
- The liver breaks down hemoglobin (that results from the breakup of dead red blood cells), creating metabolites (bilirubin and biliverdin) that are excreted through bile.

- The liver breaks down toxic substances and most medicinal products in a process called drug metabolism. This sometimes results in toxication, when the metabolite is more toxic than its precursor.
- The liver converts ammonia to urea.
- The liver stores a multitude of substances, including glucose in the form of glycogen, vitamin B12, iron, and copper.
- In the first trimester fetus, the liver is the main site of red blood cell production. By the 32nd week of gestation, the bone marrow has almost completely taken over that task.
- The liver is responsible for immunological effects- the reticuloendothelial system of the liver contains many immunologically active cells, acting as a 'sieve' for antigens carried to it via the portal system.

10.4 Diseases of the liver

Many diseases of the liver are accompanied by jaundice caused by increased levels of bilirubin in the circulatory system. The most important diseases of the liver are the following:

- Hepatitis, inflammation of the liver, caused mainly by various viruses but also by some poisons, autoimmunity or hereditary conditions.
- Cirrhosis is the formation of fibrous tissue in the liver, replacing dead liver cells. The death of the liver cells can for example be caused by viral hepatitis, alcoholism or contact with other liver-toxic chemicals.
- Hemochromatosis, a hereditary disease causing the accumulation of iron in the body, eventually leading to liver damage.
- Cancer of the liver (primary hepatocellular carcinoma or cholangiocarcinoma and metastatic cancers, usually from other parts of the gastrointestinal tract).
- Wilson's disease, a hereditary disease which causes the body to retain copper.

- Primary sclerosing cholangitis, an inflammatory disease of the bile duct, autoimmune in nature.
- Primary biliary cirrhosis, autoimmune disease of small bile ducts
- Budd-Chiari syndrome, obstruction of the hepatic vein.
- Gilbert's syndrome, a genetic disorder of bilirubin metabolism, found in about 5% of the population.
- Glycogen storage disease type II. The build-up of glycogen causes progressive muscle weakness (myopathy) throughout the body and affects various body tissues, particularly in the heart, skeletal muscles, liver and nervous system. Clinically, liver failure carries a high mortality, about 90%.

The impairment or loss of liver functions such as detoxification, metabolism, and regulation lead to life-threatening complications, including kidney failure, encephalopathy (‘hepatic coma’), cerebral edema, severe hypotension, and susceptibility to infections culminating in multiple organ failure in patients with acute liver failure (ALF). The only established effective treatment for such patients is liver transplantation, either from living or non-living donors.

However, currently, one-third of these patients die while waiting for transplants because of the organ shortage. A considerable amount of work has been done over many years to develop effective liver support devices in order to bridge the failing liver to transplantation or as a bridge to recovery, as liver failure is potentially reversible because of liver regeneration. The development of these devices has followed two different strategies. The first approach is based on detoxification functions using membranes and adsorbents. The second approach comprises biological devices using viable cells.

10.5 State of development

10.5.1 Hemodialysis

At present, hemodialysis is the standard therapy for end stage renal disease. The artificial kidney has had a profound influence on the development of the artificial liver. In 1958, Kiley et al. reported the first use of hemodialysis to treat liver failure caused by liver cirrhosis. Five patients suffering from ammonia intoxication were treated with hemodialysis.

This membrane removed many of the higher molecular weight molecules associated with encephalopathy, up to a molecular weight of 15000. The recovery from encephalopathy was statistically significant; however, the changes in survival rate were not. Removal of these substances was not able to affect the survival of patients with liver failure, although hemodialysis did work well on renal failure associated with liver failure.

10.5.2 Hemodiafiltration

Based on the assumption that mid-sized molecules are responsible for hepatic coma in patients with fulminant hepatic failure (FHF), a hemodiafiltration (HDF) method using a high-performance membrane such as a large-poresized polymethylmethacrylate (PMMA) membrane was developed in 1986. In a retrospective study, patients showed complete recovery from deep coma and long-term survival in cases of severe FHF.

To efficiently remove middle-molecular-weight substances such as hepatic toxins and to minimize the adverse effects associated with plasma exchange, Nitta et al. have developed a combination of slow plasma exchange in combination with high-flow continuous hemodiafiltration (See Figure 1).

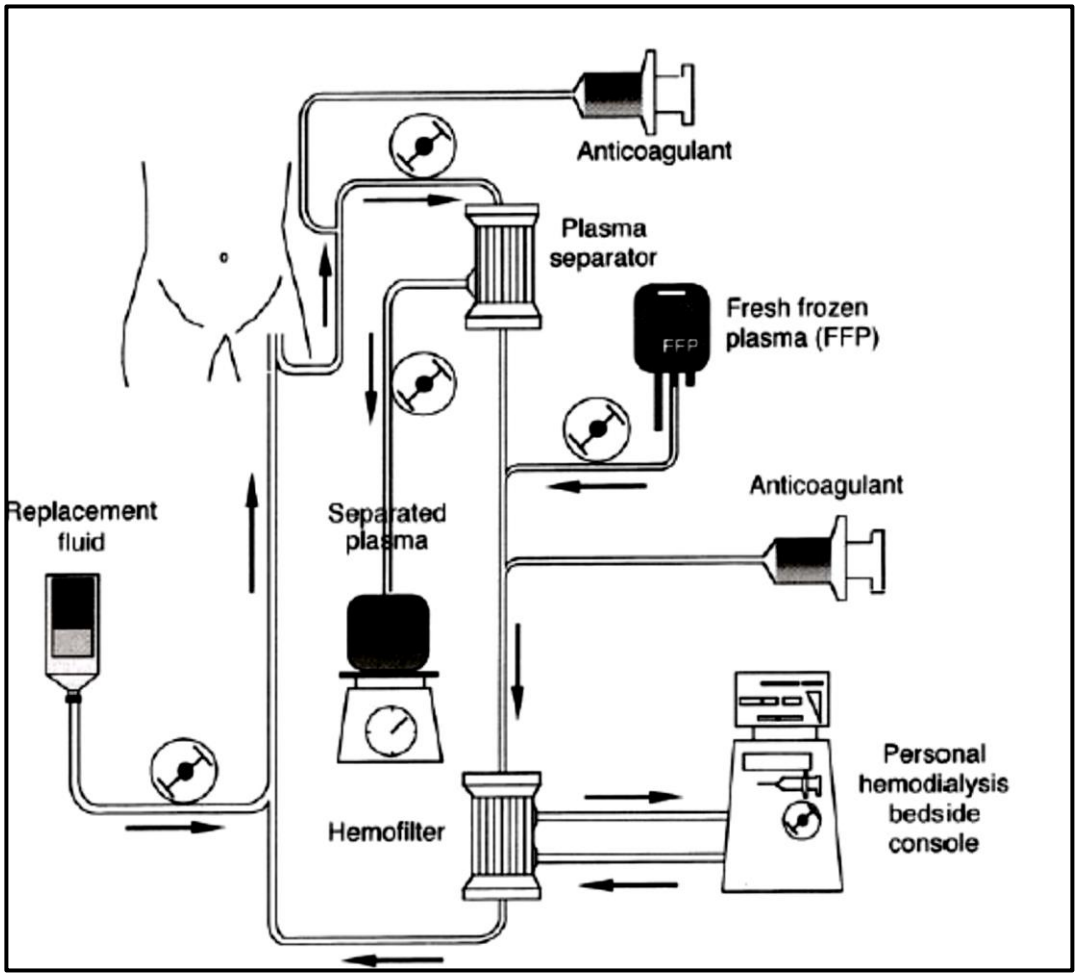


Figure 1. Flow diagram and operational conditions of slow plasma exchange (PE) plus high-flow continuous hemodiafiltration (CHDF).

10.5.3 Cryofiltration

Cryofiltration is an extracorporeal immune modulation technique originally introduced by Malchesky et al. Cryofiltration for plasma treatment uses two filters, a plasma separator and a cryofilter (see Figure 2). The patient's blood is led to the first filter in which the blood is separated, and the plasma is cooled in the presence of heparin in a heat exchanger. The plasma is then filtered through a second filter made of cellulose-diacetate.

The main indications are immune complex diseases including rheumatoid arthritis, systemic lupus erythematosus, polymyositis, primary biliary cirrhosis, and chronic

rejection of a graft kidney. The advantage of this modality is that there is no need for massive volumes of plasma, which may cause infection. Cryofiltration combined with plasma-exchange therapy has improved persisting cholestasis, hyperbilirubinemia, and hepatic coma. However, the survival rate did not improve. The mechanism of the effects of cryofiltration may be attributed to the improvement of hepatic microcirculation caused by a reduction of plasma viscosity and activation of the bile pathway by the reduction of cryogel, including immunoglobulin and immune complexes.

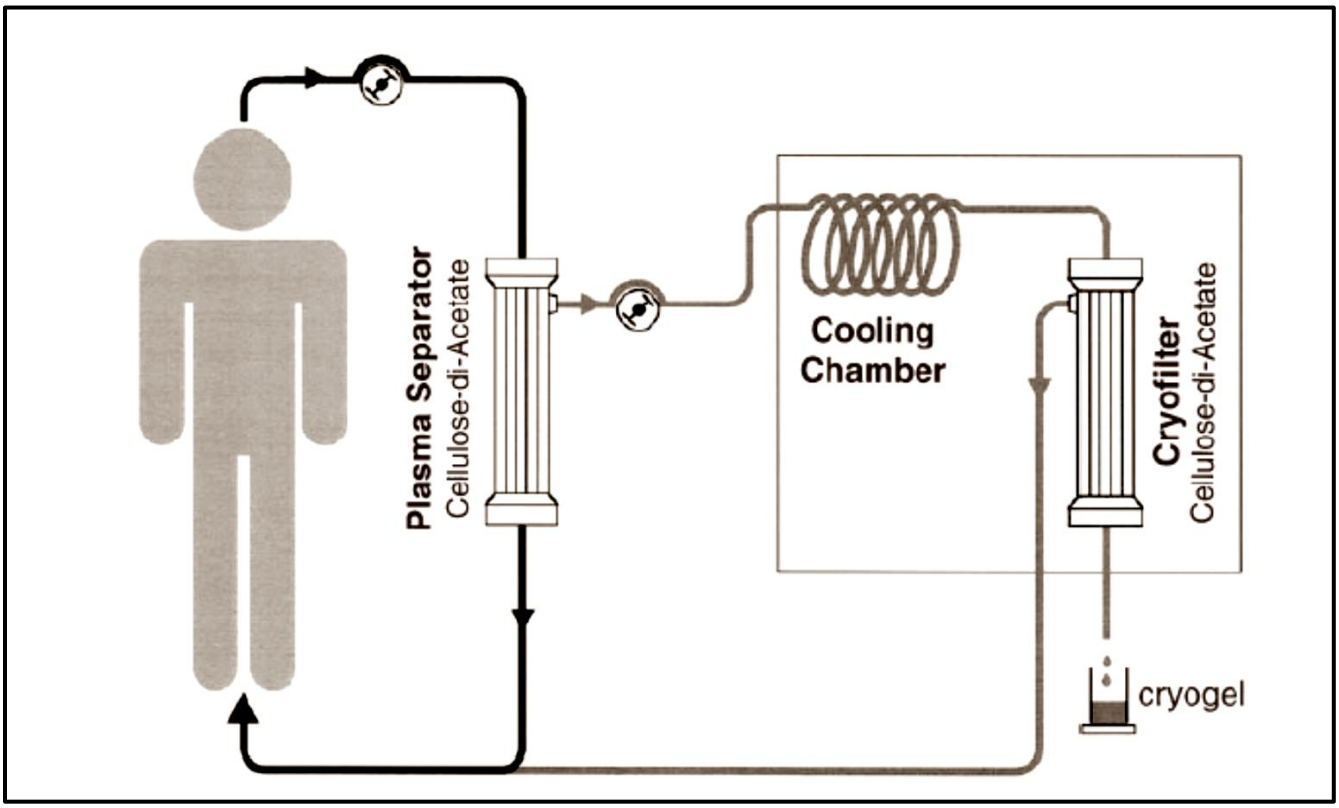


Figure 2: Cryofiltration system incorporating plasma separation, plasma cooling, and membrane filtration to remove cryogel.

10.5.4 Molecular adsorbent recycling system (MARS)

Many therapeutic procedures based on removal of toxins failed to improve patient survival. The reason for therapy failure is that most of these techniques predominantly focus on removal of water-soluble substances, whereas the accumulation of protein bound substances is unaffected. In addition, unintended removal of various growth factors delays

the process of liver regeneration. MARS system combines hemodialysis against albumin dialysate with a conventional dialysis procedure.

MARS has been shown to remove water-soluble and albumin bound low- and middle molecular-weight toxins with high selectivity as a result of the use of a high-flux membrane, such as a polysulfone membrane. Moreover, it has an additional dialysis component for removal of water-soluble toxins. MARS uses a hollow-fiber dialysis module in which the patient's blood is dialyzed across an albumin-impregnated polysulfone membrane with a cut-off of 50 kDa while maintaining a constant flow of 600ml of 20% albumin as dialysate in the extra capillary compartment (see Figure 3).

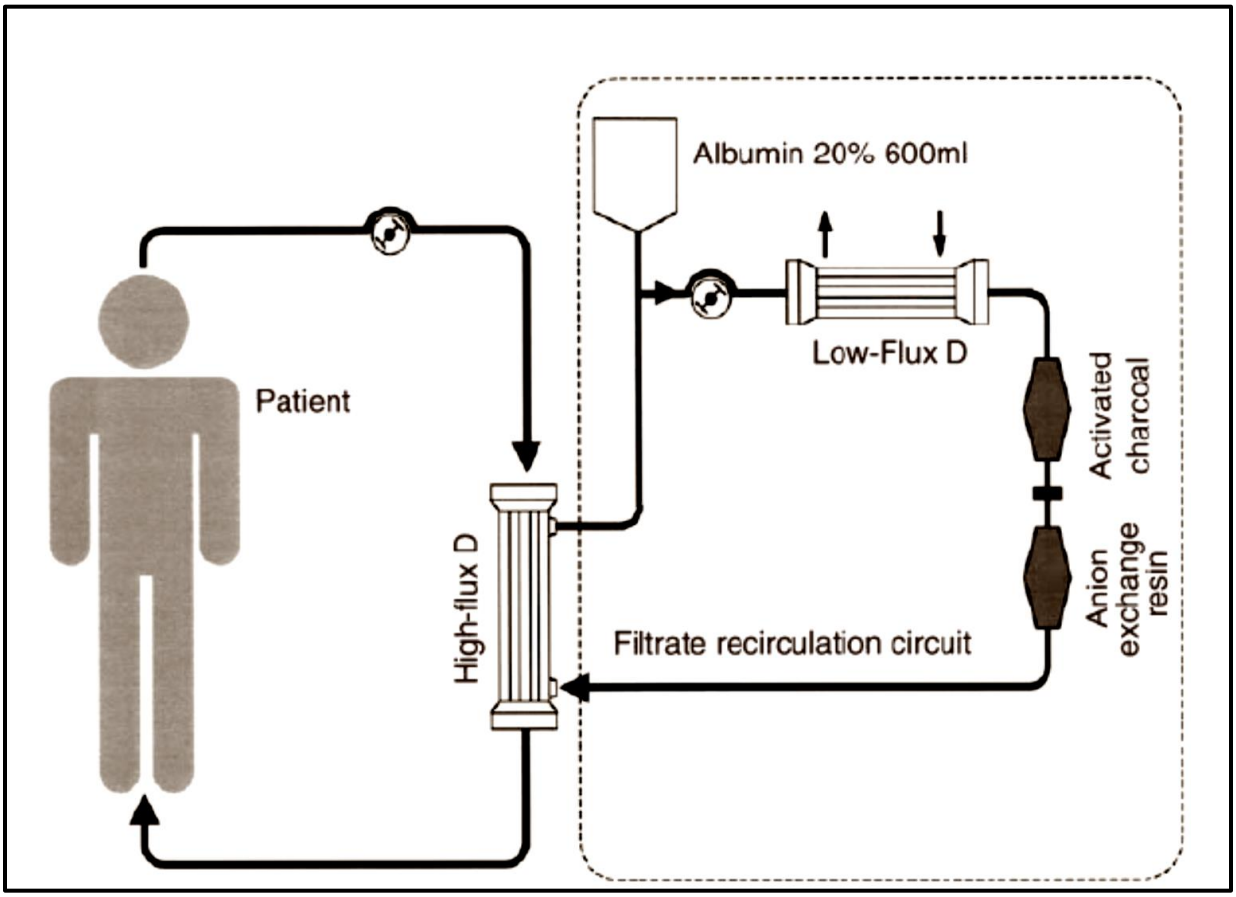


Figure 3: The molecular adsorbent recycling system (MARS) apparatus consists of a hollow fiber dialysis module in which the patient's blood is dialyzed across an albumin impregnated high-flux polysulphone membrane (MARSFlux), while at the same time maintaining a constant flow of albumin-rich (usually 20%) dialysate in the extra

capillary compartment. The adsorbed toxins from the binding sites on the membrane pass to the albumin binding sites in the dialysate. The dialysate is then passed through a column through which conventional dialysis/ filtration is performed across a low-flux membrane, and then perfused successively over an activated charcoal column and an anion exchange resin column to remove the albumin-bound toxins, and thus regenerate the dialysate.

MARS achieves high clearances for water-soluble substances (e.g. ammonia, cytokines, creatinine, urea) because of high dialysate flow rates as well as high clearance for albumin bound substances (such as bilirubin, bile acids, and nitric oxide as an endogenous vasodilator). The advantage of MARS is that it is easy to use and inexpensive compared to bioartificial devices or standard medical therapy.

10.5.5 Fractional plasma separation and adsorption (FPSA)

The Prometheus system is a FPSA method combined with high-flux hemodialysis (see Figure 4). FPSA uses an albumin permeable membrane with a cut-off of 250 kDa. Both albumin and albumin-bound toxins cross the membrane and pass through special adsorbers containing a neutral resin adsorber and anion exchanger. Prometheus treatment significantly improved serum levels of conjugated bilirubin, bile acids, ammonia, cholinesterase, creatinine, urea, and blood pH.

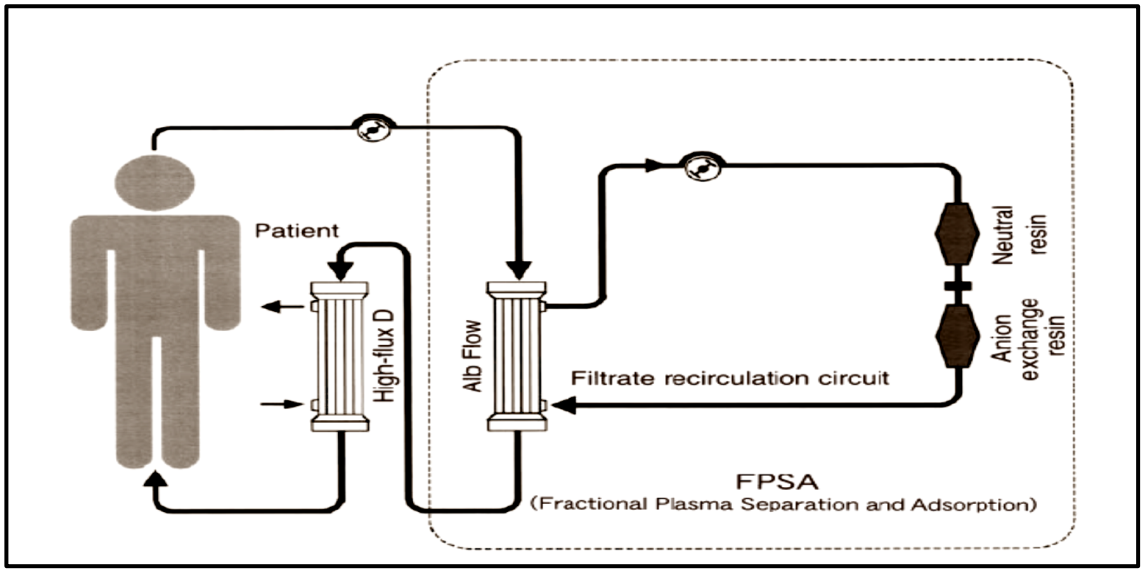


Figure 4: The circuit of the Prometheus system. Blood flows through a special albumin-permeable filter, AlbuFlow, in which the patient's own albumin (Alb) is separated from the blood. The albumin is then perfused through two adsorber cartridges. The purified albumin then reenters the blood stream. Afterwards the blood passes through a high-flux dialyzer.

10.6 Challenges:

In addition to the variable nature of the clinical effects of liver failure, differences exist in design of the bioreactors. Therefore, their clinical performance cannot readily be predicted based on parameters like cell mass, mass transfer rate, mode of oxygenation, treatment duration, direct/indirect contact between plasma and cells and flow/exchange rates.

Based on the current clinical experiences, three problems have been mentioned in relation to the biological and physical limitations of bioreactor design:

- exchange capacity: normal blood flow through the liver is about 1.5 l/min, in a bioartificial device flow rates are limited to 0.1 to 0.3 l/min. because of technical and rheological reasons.
- cell mass: in living donation transplantation the transplanted cell mass should at least be 40% of the ideal liver mass of the recipient to meet the metabolic needs otherwise the graft may fail. Currently, cell mass in BALs ranges from 50-500 g, which is at most about one third of the weight of an adult liver.
- related to the problem of cell mass: a cell source that can supply large amounts of hepatocytes of good quality. Discarded livers for transplantation may serve to this end, but their number is limited (about 1 in 5 explanted organs) and their cell quality is not optimal due to preservation and isolation processes.

The causes leading to liver failure are various. In clinical studies focusing on liver failure (treated with bioartificial devices) it can be difficult to recruit a homogeneous and

large enough group of patients. In case multicenter trials are conducted, care must be taken to harmonize the ‘standard’ level of care in the control group.

From the clinical experiences of recent years and the adaptations in design of the newer BALs one can identify three trends, partly solving the abovementioned problems:

1) cell mass is increasing (towards 20 billion cells),
2) immunological barriers are not a requirement (short term contact with xenogeneic cells does not induce important immunological complications),
3) hepatocytes are being cultured as organoids, i.e. having appropriate cell cell interactions, including interaction with non-parenchymal cells.