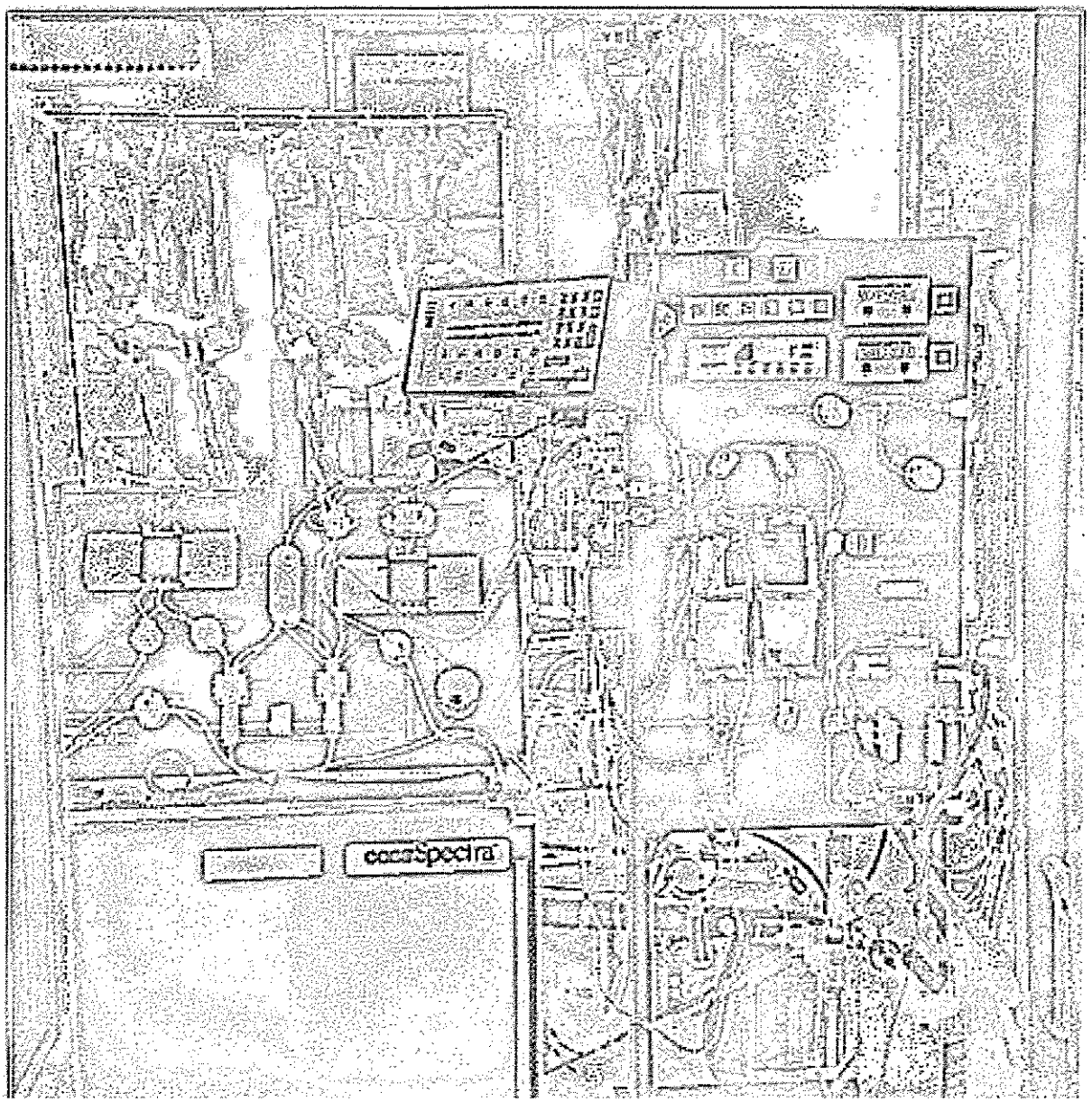

Alternative and standard treatments of acute hepatic failure

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1. Summary

Liver failure is related with high mortality[1]. Liver transplantation has emerged as an effective therapy for patients with hepatic failure who do not respond to standard management. However, due to the donor organ shortage and the urgent need for transplantation, many patients die before transplantation. Some patients do not even survive after transplantation, primarily because of intracranial hypertension and subsequent brain stem herniation. Different liver assist devices (LAD) have evolved, treating patients with severe liver failure until either transplantation or spontaneous recovery. However, the efficacy of these devices despite promising results is uncertain as compared to standard treatment.

This text looks into the world of liver assist devices and the literature on the subject. The assumptions from the literature are considered; the requirements of liver assist devices are outlined. The differences between the non-biological and biological LADs are considered. The former are applied for patients with acute-on-chronic liver failure, while the latter are used for patients with acute liver failure. Further, the different aspects of hepatic failure are considered. Additionally, different approaches with their advantages and disadvantages are discussed, for example the use of human versus porcine hepatocytes.

The intention of the text is not to present a meta-analysis of the different approaches, but rather an updated overview of the current trials so far presented. Furthermore, the various systems are not ranked accordingly to their efficacy, due to limitations of comparable end-points of the trials presented so far. All the considered devices and their trials have limitations such as small sample sizes and the lack of good end-points. Thus, the lack of randomized, prospective, large trials with comparable end-points are needed to establish the efficacy of these devices.

2. Introduction

Severe liver failure is associated with high mortality[1]. Liver transplantation has emerged as an effective therapy for patients not responding to standard therapy. However, because of the donor organ shortage and urgent need for transplantation, many patients die before they can be transplanted. Other patients do not survive after transplantation, primarily because of intracranial hypertension. The estimated incident of acute hepatic failure in the United States is 2000, representing approximately 0.1 % of all deaths[2]. The survival rate correlate closely with age, in patients aged between 10 and 40 years, the survival rate varies between 30-35%. In patients aged younger than 10 or older than 40 years, the survival rate is less than 10%[3, 4, 20].

There are several definitions of liver failure. The term fulminant hepatic failure was first defined by Trey[5] and defined as follows: "A potential reversible condition, the consequence of severe liver injury, with an onset of encephalopathy within 8 weeks of the appearance of the first symptoms and in the absence of pre-existing liver disease".

The standard supportive treatment is highly advanced and a major clinical challenge, calling for particular awareness. The focuses are on management of respiration, hemodynamic management and intracranial pressure monitoring. Treatment is directed towards early recognition of the complications and general supportive

measures. The only proven therapy for those who are unlikely to recover is liver transplantation.

Different liver assist devices (LAD) have been evolved, in order to treat patients with severe liver failure until they can be either transplanted or recover spontaneously. However, the improved success and efficacy of these devices are uncertain compared to conventional treatment, despite promising results.

In 1956 Kiley *et al* were one of the first to use hemodialysis in hepatic coma[6] and Sorrentino initiated the term "artificial liver"[7]. Schechter *et al* tried out

3. Liver structure and function

The liver is the largest organ of the body, weighing 1 to 1.5 kg or representing 1.5 to 2.5% of the lean body mass. The size and shape of the liver vary and generally match the general body shape—long and lean or squat and square. The liver is held in place by ligamentous attachments to the diaphragm, peritoneum, great vessels, and upper gastrointestinal organs. It receives a dual blood supply; approximately 20% of the blood flow is oxygen-rich blood from the hepatic artery, and 80% is nutrient-rich blood from the portal vein arising from the stomach, intestines, and spleen.

The greater part of cells in the liver is hepatocytes, which constitute two-thirds of the mass of the liver. The remaining cell types are Kupffer cells, stellate cells, endothelial cells, and bile ductular cells. Kupffer cells are members of the reticuloendothelial system, while stellate cells are fat-storing cells.

As suggested above, there are 5 different types of cells in a liver:

1. Hepatocytes
2. Kupffer cells
3. Stellate cells
4. Endothelial cells
5. Bile ductular cells

hemofiltration, using cationic resins a couple of years later[8]. However, the field remained undeveloped until the 1970s and 80s, when several improvements in cell harvesting and culturing made hepatocytes more accessible for experimental use. The first LAD based on isolated, living cells was first investigated by Matsumura, utilizing cryopreserved rabbit hepatocytes in 1987[9]. Hepatocytes of human origin human became more available with the hepatoblastoma derived cell line; Sussman *et al* applied this cell line for the first time in a LAD in 1993[10]. The use of cell lines, or immortalized cells, versus primary cells and their use will be closer discussed.

Examined by light microscopy, the liver appears to be organized in hexagonal lobules, with portal areas at the periphery and central veins in the midpoint of each lobule. However, from a functional point of view, the liver is organized into acini, with both hepatic arterial and portal venous blood entering the acinus from the portal areas and then flowing through the sinusoids to the terminal hepatic veins. The advantage of viewing the acinus as the physiologic unit of the liver is that it helps to explain the morphologic patterns of many vascular and biliary diseases not explained by the lobular arrangement.

Portal areas of the liver consist of small veins, arteries, bile ducts, and lymph vessels organized in a loose stroma of supporting matrix and small amounts of collagen. Blood flowing into the portal areas is distributed through the sinusoids, passing from zone 1 to zone 3 of the acinus and draining into the terminal hepatic veins, called central veins. The sinusoids are lined by unique endothelial cells that have prominent fenestrae of variable size, allowing the free flow of plasma but not cellular elements. The plasma is thus in direct contact with hepatocytes in the sub-endothelial space of Disse. The zones 1 to 3 represent metabolic regions increasingly distant from the blood supply. Figure 1 illustrate these zonation and define acinus and lobules.

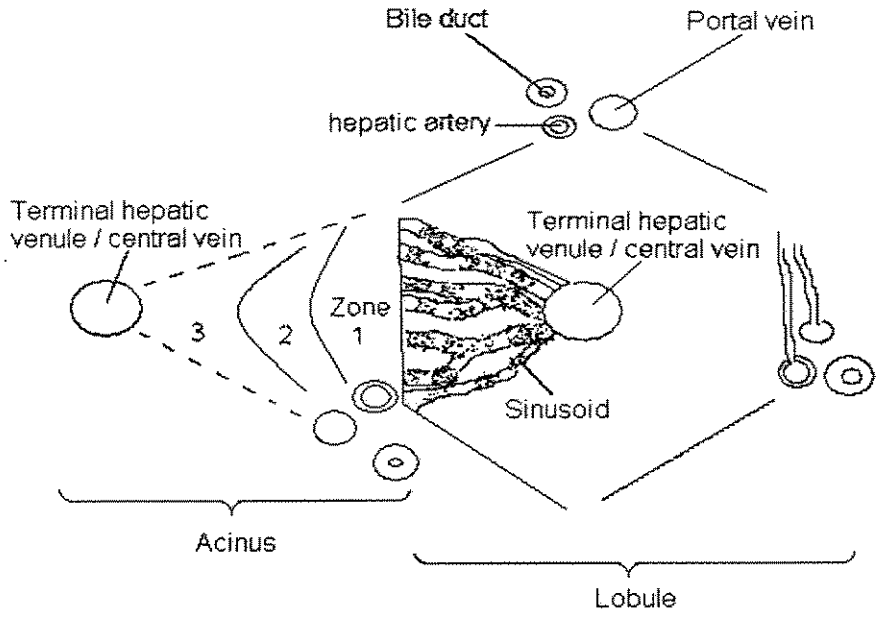


Figure 1: The functional organization of a liver acinus with its zonations, and lobule. (Based on drawing from J. Crawford. The liver and the biliary system. Robbins, Kumar, Cotran. "Basic Pathology", sixth edition. United States of America, Saunders, 1997.)

Hepatocytes have distinct polarity. The basolateral side of the hepatocyte lines the space of Disse and is richly lined with microvilli; it demonstrates endocytotic and pinocytotic activity, with passive and active uptake of nutrients, proteins, and other molecules. The apical pole of the hepatocyte forms the cannicular membranes through which bile components are secreted. The canniculi of hepatocytes form a fine network, which fuses into the bile ductular elements near the portal areas.

Kupffer cells usually lie within the sinusoidal vascular space and represent the largest group of stationary macrophages in the body. The stellate cells are located in the space of Disse but are not usually prominent unless activated, when they produce collagen and matrix. Red blood cells stay in the sinusoidal space as blood flows through the lobules, but white blood cells can migrate through or around endothelial cells into the space of Disse and from there to portal areas, where they can return to the circulation through the lymphatic system.

Hepatocytes perform numerous and vital roles in maintaining homeostasis and health. These functions include:

- The synthesis of most essential serum proteins. These proteins include albumin, carrier proteins, coagulation factors, different growth and many hormonal factors.
- The production of bile and its carriers: bile acids, cholesterol, lecithin and phospholipids.
- The regulation of nutrients: glucose, glycogen, lipids, cholesterol and amino acids.
- Metabolism and conjugation of lipophilic compounds like bilirubin, cations and numerous drugs, for excretion in the bile or urine.

The wide variety of functions of the hepatocytes differs to its location in the liver. The hepatocytes in the above mentioned metabolic zones execute unlike metabolic assignments according to blood supply. This zonation is of considerable metabolic consequence, because a lobular gradient of activity exists for many hepatic

enzymes. The periportal and centrilobular hepatocytes express high levels of urea cycle enzymes and low levels of glutamine synthetase while the perivenous hepatocytes represent the opposite. The hepatocytes are also influenced by shear stress. If the shear stress is low, albumin and urea synthesis dominate, while at high shear stress, cytochrome P450 activity predominate.

In normal animals and humans, liver regeneration is a tightly regulated process of both hypertrophy and hyperplasia involving different liver cell populations and a finely tuned regulation and feed-back regulation between growth factors, cytokines, extracellular matrix components and other affecting substances. Conditions and factors that encourage latent tissue to move into a regenerative cycle from G_0 to G_1 and S phases are currently under investigation. Several substances have been proposed to be important in promoting tissue regeneration, for instance hepatocyte growth factor, epidermal growth factor, transforming growth factor α, interleukin-6, insulin, noradrenalin and tumour necrosis factor α.

Experimentally, there is solid evidence suggesting that all the above-mentioned substances in some way contribute to regeneration of hepatocytes. However, hepatocyte growth factor plays a key role in regeneration. On the other hand, until today there are not known any particular substances that completely prevent liver generation[11]. It is therefore likely that the initiation of the process and the complete picture of the regeneration process are still unknown[12].

4. Definitions and characteristics of hepatic failure

Acute hepatic failure occurs in a previously healthy liver, while an acute exacerbation of an already chronic liver disease is called acute-on-chronic hepatic failure. Although severity and progression of development vary, clinically they manifest similarly, with encephalopathy, jaundice, hepatorenal syndrome and circulatory changes[18]. Probably is the pathophysiological basis the same: The liver starts to dysfunction and a subsequent accumulation of toxins result in clinical and paraclinical manifestations. Hence, to use the term hepatic failure, organ dysfunctions like encephalopathy with or without hepatorenal syndrome should be present[13]. The acute hepatic failure is potentially reversible, while a chronic progression typically is not.

While there are many causes of liver disease, they generally present clinically in a few distinct patterns, usually classified as either:

1. Hepatocellular, or
2. Cholestatic/obstructive

Acute viral hepatitis
Acute drug-induced hepatitis
Acute hepatitis due to poisoning
Ischemic liver cell necrosis
Obstruction of the hepatic veins
Massive malignant infiltration of liver
Wilson's disease
Micro vesicular steatosis
Autoimmune chronic active hepatitis
Reactivation of chronic hepatitis B
Hyperthermia
Liver transplantation
Partial hepatectomy

Figure 2: Causes of fulminant or subfulminant hepatic failure. (Bernau, et al: Fulminant and subfulminant liver failure: definitions and causes. Semin. Liver dis. 1986, 6 (2) 98.)

In hepatocellular diseases, such as viral hepatitis or alcoholic liver disease, features of liver injury, inflammation, and necrosis predominate. In cholestatic diseases, such as gallstone or malignant obstruction, primary biliary cirrhosis, many drug-induced liver diseases, features of inhibition of bile flow predominate. The pattern of onset and severity of symptoms can rapidly suggest a diagnosis, particularly if major risk factors are considered, such as age and sex of the patient, and a history of exposure or risk behaviours[14]. See figure 2 for different causes of fulminant or subfulminant hepatic failure.

Hepatic failures arise when the liver as an organ ceases to function normally, in other words when the functional liver cell mass falls below a critical level. Synthetic functions, detoxification, and metabolic regulation may be weakened at different degrees and may influence each other. Among others, the body as a result of this is exposed to several substances and toxins, which the liver usually detoxifies, and the failure reaches systemic influence. The most important toxins of hepatic failure are strongly protein-bound or lipid-bound, including bilirubin, endotoxins, TNF-α, IL-1β and IL-6[15]. Some of the albumin-bound toxins are seen in figure 3.

Aromatic amino acids
Bile acids
Bilirubin
Digoxin-like substances
Endogenous benzodiazepines
Indols
Mercaptans
Middle- and short-chain fatty acids
Nitric oxide
Phenols
Prostacyclins
Tryptophan

Figure 3: Some of the endogenous albumin-bound toxins accumulating in hepatic failure (Sen, Jalan, Williams. Extracorporeal albumin dialysis in acute-on-chronic liver failure: Will it stand the test of time? Hepatology 2002; 36; 4; 1014-16).

4.1 Pathophysiological characteristics of hepatic failure

Pathophysiologically, there are two aspects that are important in hepatic failure:

- Loss of hepatocellular function
- Shunting of blood around the liver in portosystemic connections.

All liver diseases may end in hepatic failure as the most severe consequence. The hepatic failure may be the end-stage of a developing disease in chronic progression with or without prior disease. Hepatic failure may appear when 80 to 90 % of the capacity of the liver is destroyed. In this situation, the ability to regenerate liver parenchyma is reduced, possibly because the remaining vital tissue is occupied doing crucial functions for sustaining life.

4.2 Morphological characteristics of hepatic failure

In many cases, intercurrent disease, such as gastrointestinal bleeding, systemic infection, electrolyte disturbances, surgery or heart failure, tips the balance towards failure when the functional reserve is uncertain. At its most severe, an acute hepatic failure will lead to multiple organ failure and subsequent death within months in approximately 80%[16, 3]. As mentioned, hepatic failure can result from a variety of causes, but morphologically hepatic failure can be subdivided into 3 categories[17]:

1. Ultra structural lesions that do not produce hepatocyte necrosis, but the hepatocytes are dysfunctional. One example is tetracycline toxicity.
2. Chronic liver disease, with hepatic failure as the end stage of the disease. There are several causes of cirrhosis; in the developed world alcohol abuse is the most common.
3. Hepatic necrosis, often caused by acute viral hepatitis and drugs.

4.3 General clinical characteristics

The varieties of liver diseases cause either acute or chronic hepatic failure, depending on the amount of time the liver takes to fail. Hepatic failure is defined differently according to their sources. One definition define hepatic failure as acute when the duration is less than 4 weeks, and subacute when the duration is between 4 weeks and 6

month[18]. Acute hepatic failure is the result of extensive hepatocyte death over the course of days or weeks, the result of either an acute process developing in the absence of pre-existing liver disease or an acute exacerbation of a chronic underlying process. The acute exacerbation is called acute-on-chronic hepatic failure. Further definitions of hepatic failure and their references are seen in figure 4. Additionally, some clinical signs and their therapy will be mentioned when discussing standard treatment.

Acute liver failure:	-Acute liver disease, with prothrombin time or factor V less than 50% of normal
Fulminant hepatic failure	-Acute liver failure with hepatic encephalopathy, developing less than 2 weeks[1] (or 8 weeks[2, 3]) after onset of icterus[1] (or illness[2, 3]).
Subfulminant hepatic failure / late onset hepatic failure	-Acute liver failure with hepatic encephalopathy, developing from 2 weeks[1] (or 8 weeks[2, 3]) to 3 months[1] (or 6 months[2, 3]) after onset of icterus[1] (or illness[2, 3])

1. Bernau J, *et al*: Fulminant and subfulminant liver failure: definitions and causes. Semin Liv Dis 1986;6:97
2. Trey C, Davidson LS: The management of fulminant hepatic failure. Progress in liver diseases, vol III. Editors: Popper Schaffer. Grune and Stratton 1970.
3. Gimson AES *et al*: Clinical and prognostic differences in fulminant hepatitis type A, B, and non-A, non B. Gut 1983; 4: 1194.

Figure 4: Different definitions of hepatic failure and references.

There are numerous clinical characteristics of hepatic failure as seen in figure 5. Jaundice is the hallmark symptom of liver disease and perhaps the most reliable marker of severity. Jaundice appears when serum bilirubin levels rise above 2.0-2.5 mg/dL. Signs of advanced liver disease include muscle-wasting and weight loss as well as hepatomegaly, bruising, ascites, and oedema.

Icterus
Hypoalbuminemia
Coagulopathy
Dissiminated intravascular coagulation
Hyperammonemia
Fetor hepaticus
Increased serum levels of hepatic enzymes: LDH, ALAT, ASAT, GGT
Gynecomastia, testicular atrophy, palmar erythema, spider angiomas
Hepatic encephalopathy
Hepatorenal syndrome
Coma

Figure 5: Some clinical and para-clinical characteristics of hepatic failure.

Ascites refers to collection of excess fluid in the peritoneal cavity. Because of reduced synthesis of albumin in the liver, hypoalbuminemia with consequent oedema emerge. A coagulopathy is also a result of reduced synthesis function of the clotting

factors II, VII, IX and X, as measured with the international normalized ratio (INR).

Fetor hepaticus is a characteristic, sweet and sour-like smell detectible in exhaled breath. It originates from methionine and other sulphur-containing compounds normally metabolised by the liver. Hyperoestrogenemia leads to vasodilatation, seen as palmar erythema and spider angiomas. The hyperoestrogenemia is due to reduced hepatic metabolism of oestrogen and reduced hepatic synthesis of sexual hormone binding globulin, SHGB. Additionally, the production of androgens is often reduced in the gonads due to altered blood supply. Hyperammonemia is a result of reduced urea cycle function in the liver. Other signs may be hypoglycaemia and ascites, which are prominent in chronic liver disease such as cirrhosis.

Other signs of advanced liver disease include umbilical hernia from ascites, prominent veins over the abdomen, and dilated abdominal veins, which consists of collateral veins seen radiating from the umbilicus and resulting from the recanulation of the umbilical vein. Widened pulse pressure and signs of a hyperdynamic circulation can occur in patients with cirrhosis as a result of fluid and sodium retention, increased cardiac output, and reduced peripheral resistance. Several skin disorders and changes occur commonly in liver disease.

The typical symptoms of cirrhosis are oedema and ascites, which, together with dilated periumbilical veins, suggests cirrhosis and extensive portal collateral circulation. Oesophagus varices may as well be present. When cirrhosis is suspected, one should look for gynecomastia, testicular atrophy, and diminished axillary or pubic hair. Additionally, in the cirrhotic patient, one should look for more generally signs of liver disease discussed above, like jaundice, pallor due to anaemia, significant cachexia, especially of the extremities.

4.4 Hepatic encephalopathy and hepatorenal syndrome

Hepatic encephalopathy is disturbed function of the brain and the main sign is reduced consciousness, and is considered to be a result from excretion of toxins normally removed by the liver. Clinically, the encephalopathy is often evident in acute hepatic failure, but more rarely in chronic cases of liver failure. Patients with acute hepatic failure and deep encephalopathy develop brain oedema, a complication that may lead to brain stem herniation and death. The hepatic encephalopathy, which will be considered later, is categorized into 4 stages[19].

There are three major hypotheses regarding the pathophysiology of fulminant hepatic encephalopathy:

- Release of toxic substances from the necrotic liver
- Cerebral accumulation of glutamine that leads to astrocyte swelling
- Disruption of the blood-brain barrier and cerebral hyperaemia

However, none of these seems to play a primary role in the pathogenesis of cerebral oedema, according to a large amount of clinical and experimental evidence. So, the pathogenesis of cerebral oedema and the subsequent raise of intracranial pressure are not fully understood[20]. On the other hand, in a recent study, ammonia was shown to be a strong pathogenic role in brain oedema[4] and many tend to believe that the theory about cerebral accumulation of glutamine is more central than the other hypotheses in the development of encephalopathy.

The failing liver is the basis for the theory of cerebral accumulation of glutamine: The portosystemic shunting of blood and loss of hepatocellular function expose the brain to an altered blood content, which leads to elevated ammonia level. In the brain, the only site for ammonia detoxification is in the astrocytes, which form glutamine from glutamate, catalysed by glutamine synthetase. As a result, glutamine accumulates in the

astrocytes and they start to swell due to the increased oncotic pressure and a subsequent osmosis of water.

Hepatic encephalopathy is considered as a metabolic disorder of the central nervous- and neuromuscular system, and in most instances there are only minor morphologic changes like oedema and astrocytic swelling. The first signs of hepatic encephalopathy can be subtle and non-specific: A change in sleep patterns, change in personality, irritability, and mental tediousness. More developed hepatic encephalopathy is clinically seen as reduced consciousness associated with neurological signs like asterixis, which is fast, non-rhythmical movements of extremities and head with a frequency of approximately 3 per second. Other signs of encephalopathy are rigidity, flapping, hyperreflexia and changes in electroencephalogram. When cirrhosis is the cause of hepatic encephalopathy, the cirrhosis stigmata will also be present. The symptoms of cirrhosis are mentioned above. Encephalopathy and renal failure may both indicate cirrhosis.

Hepatorenal syndrome is reduced renal function, with hypoperfusion and apparently reduced perfusion pressure of the organ. The ability to concentrate urine is maintained, producing hyperosmolar urine with hyponatremia and high serum creatinin levels. Despite intravenous fluid treatment, the perfusion and the perfusion pressure of the kidneys are low. There are no intrinsic morphologic or functional changes in the kidneys. The pathophysiology of hepatorenal syndrome is unclear, but it is thought to be reduction of renal blood flow, especially in the cortex, which results in vasoconstriction and further decreased glomerular filtration rate. Nitric oxide and its binding to albumin which gives nitric oxide systemic action, is thought to participate in the development of hepatorenal syndrome[21]. When hepatic function is restored, kidney perfusion and performance improves.

5. Diagnosis, grading and staging of hepatic dysfunction

In most instances, a diagnosis of liver disease can be made accurately by a careful history, physical examination, and application of laboratory tests. In some instances, radiologic examinations are helpful or, indeed, diagnostic. Liver biopsy is considered the gold standard in evaluation of liver disease but is now needed less for diagnosis than for grading and staging disease.

Evaluation of patients with liver disease should be directed at:

1. Establishing the etiologic diagnosis
2. Estimating the disease severity in grading the disease
3. Establishing the disease stage

Diagnosis should focus on the category of disease, such as hepatocellular versus cholestatic injury, as well as on the specific etiologic diagnosis. Grading refers to assessing the severity or activity of disease—active or inactive, and mild, moderate, or severe. Staging refers to estimating the place in the course of the natural history of the disease, whether acute or chronic; early or late; pre-cirrhotic, cirrhotic, or end-stage.

Measurement of the activities of the hepatocytes to assess liver function is complicated by the multiplicity and variability of these functions. The most commonly used liver function tests are measurements of serum bilirubin, albumin, and INR. The serum bilirubin level is a measure of hepatic conjugation and excretion, and the serum albumin level and INR are measures of protein synthesis. Abnormalities of bilirubin, albumin, and INR are typical of hepatic dysfunction. Forthright liver failure is incompatible with life, and the functions of the liver are too complex and diverse to be subserved by a mechanical pump; dialysis membrane; or mixture of infused hormones, proteins, and growth factors.

The severity of hepatic dysfunction is determined by a clinical evaluation for the presence and severity of encephalopathy, ascites, and evidence of muscle wasting. Liver biopsy is the most accurate means of assessing stage of disease as early or advanced, precirrhotic, and cirrhotic. Staging of disease pertains largely to chronic liver diseases in which progression to cirrhosis and end-stage liver disease can occur, but which may require years or decades to develop. Clinical features, biochemical tests, and hepatic imaging studies are helpful in assessing stage but generally become abnormal only in the middle to late stages of cirrhosis. Early stages of cirrhosis are generally detectable only by liver biopsy.

5.1 The Child-Turquotte-Pugh, Model for End-Stage Liver Disease (MELD), and the King's College classifications

Cirrhosis can also be staged clinically. A reliable staging system is the modified Child-Turquotte-Pugh classification with a scoring system of 5 to 15:

- Child-Turquotte-Pugh class A: Scores of 5 and 6 being consistent with the term compensated cirrhosis.
- Child-Turquotte-Pugh class B: Scores of 7 to 9 being consistent of the term decompensated cirrhosis.
- Child-Turquotte-Pugh class C: Scores of 10 to 15 being consistent of the term serious decompensated cirrhosis.

This scoring system was initially devised to stratify patients into risk groups prior to undergoing portal decompressive surgery. It is now used to assess prognosis in cirrhosis and provides the standard criteria for listing for liver transplantation. Child-Turquotte-Pugh class B or a score of 7 is needed to be listed in the United States for liver transplantation. The Child-Turquotte-Pugh score is a reasonably reliable predictor of survival in many liver diseases and predicts the likelihood of major complications of cirrhosis such as bleeding from varices and spontaneous bacterial peritonitis. Other means of assessing stage and survival have been developed for primary biliary cirrhosis and sclerosing cholangitis, such as Mayo-Risk scores, which are somewhat more accurate but which actually rely mostly on the same measurements as the Child-Turquotte -Pugh score.

The Child-Turquotte-Pugh approach uses the graded system to assign a numerical risk on the basis of serum albumin and serum bilirubin measurements, the presence of ascites and encephalopathy, and nutritional status, see figure 6. Recently the MELD score has been proposed as an alternative to the Child-Turquotte-Pugh score. The score consists of serum bilirubin and creatinine levels, INR for prothrombin time, and the etiology of the liver disease. Efforts are underway to use the MELD score to determine organ allocation rather than the Child-Turquotte-Pugh score.

Factor	1	2	3
Serum bilirubin, μmol/L (mg/dL)	<34 (<2.0)	34-51 (2.0-3.0)	>51 (> 3.0)
Serum albumin g/L (g/dL)	>35 (>3.5)	30-35 (3.0-3.5)	<30 (< 3.0)
Ascites	None	easily controlled	poorly controlled
Neurologic disorder	None	minimal	advanced coma
INR	<1.7	1.7-2.3	>2.3

Figure 6: The Child Pugh classification of cirrhosis.

Furthermore, the King's College criteria are frequently used to determine organ allocation today[22]. The survival of non-transplanted patients who meet the criteria are lower than 15%[23]. The criteria separate between use of paracetamol or not, see figure 7.

Paracetamol
PH < 7.30 after resuscitation
or
INR > 6.5
Creatinine > 3.4 mg dL^{-1}
Stage III or IV encephalopathy

Non-paracetamol
INR > 6.5
or
Age < 10 or > 40 years
Etiology: Drug reaction
Jaundice > 7 days
INR > 3.5
Bilirubin > 17.5 mg dL^{-1}

Figure 7: The King's College criteria for determination of organ allocation.

5.2 Hepatic encephalopathy

Hepatic encephalopathy is clinically divided into four grades[14]:

- Grade I: Changes of personality and sleeping pattern.
- Grade II: In more severe cases, the term slow cerebration is used, seen as prolonged intellectual and muscular reaction time and confusion.
- Grade III: Stupor and somnolence is typical.
- Grade IV: Total loss of any reactions on stimulus i.e. coma[24]

The degree of encephalopathy is believed to be a strong predictor of outcome from acute hepatic failure. Among patients reaching stage II encephalopathy, the possibility of spontaneous recovery under standard intensive care ranges between 65 and 70%, with stage III between 40 and 50%, and with stage IV 20% or less. The above-mentioned

statistics are found in large series with patients with acute hepatic failure[25, 26, 7].

6. Standard treatment of hepatic failure

Standard supportive treatment of hepatic failure is directed towards early recognition of the complications and general supportive measures. The only proven therapy for those who are unlikely to recover is liver transplantation. Treatment is highly advanced and a major clinical challenge with focus on:

- Management of respiration
- Hemodynamic management
- Intracranial pressure monitoring

Clinically, the supportive treatment includes supportive breathing apparatus, dialysis and antibiotics. For instance, the use of Glasgow coma scale is important to control the progress of the disease. Elevated intracranial pressure is cared for routinely and cerebral oedema is aggressively treated with Mannitol and hyperventilation[27]. In patients with fulminant hepatic failure, intracranial pressure is usually monitored with either subdural or epidural transducers, for instance a probe. The risks of placement of intracerebral pressure transducers are hemorrhage and infection, but the benefit appears to outweigh this risk. If necessary, the patient will receive ulcer prophylaxis and dialysis.

Hypoglycemia is a common complication of severe acute hepatic failure. Hence, blood glucose levels should be closely monitored. The pathophysiology of hypoglycemia is multifactorial, including impaired hepatic glucose release, impaired hepatic glucogenesis, and elevated serum insulin levels. Treatment of hypoglycemia may require infusion of hypertonic glucose or 10% dextrose. A sensible ambition is to keep blood glucose levels at 60-200 mg/dL. Additionally, the patient requires a caloric supplement of 35-50 kcal/kg for resting metabolic demands.

In acute hepatic failure there may be various abnormalities of coagulation. Decreased levels of factors II, V, VII, IX and X account for the prolongation of the prothrombin time. The clinical condition and prognosis are best determined by serial measurements of INR and factor V levels. Thus, infusion of fresh frozen plasma is indicated only for bleeding or at invasive procedures. A coagulopathy predisposes for bleeding from the gastrointestinal tract.

The goal of intracerebral pressure monitoring is to maintain the pressure at less than 20 mm Hg; a persistent pressure greater than 40 mm Hg and refractory to treatment precludes orthotopic liver transplantation. In addition, the cerebral perfusion pressure should be maintained above 50 mm Hg.

Oral lactulose therapy is a standard of care in toxin removal, and its primary function is to reduce production of ammonia in the gut, which is sometimes pathologically elevated. However, the effect of lactulose therapy is limited, because it does not have an effect in all patients. The toxin removal capacity is narrow and the therapy has severe side effects like prolonged diarrhoea.

Patients with acute hepatic failure may experience systemic hyperdynamic circulation, similar to patients with chronic hepatic illnesses. This is due to loss of autoregulation of vasogenic tone with reduced systemic vascular resistance[28]. The hyperdynamic circulation leads to subsequent compensatory increased cardiac output and reduced peripheral resistance. Eventually, this leads to lowered blood pressure. The mechanism behind compensatory hypotension[29] is not fully known. Patients with acute hepatic failure receive vasopressor therapy, like nor-epinephrine intravenously, to sustain sufficient arterial pressure[30].

Finally, the patients with acute hepatic failure are at increased risk of various bacterial as well as fungal infections. Infection make transplantation contra-

indicated before recovery. The predominant infective organisms are Staphylococcus aureus, gram-negative bacteria and Candida albicans. Aggressive medication with antibiotics on indication or prophylactic treatment is critical for survival. Prophylactic anti-fungal treatment is regularly used in hepatic encephalopathy, due to high levels of morbidity and incidence.

6.1 Hemodialysis

Initially, the intention of hemodialysis was a treatment mode for end-stage renal disease where the blood of the patient flows outside the body through disposable bloodlines into a special filter, the dialysis unit. Dialysis is any process that changes the concentration of solutes in the plasma by exposure to a second solution, the dialysis liquid, across a semi-permeable membrane. The purification process relies upon convective pressure or diffusion models. Convective pressure means high ultrafiltration fluxes in an attempt to remove the small molecular weight solutes. Dialysis solution carries away waste products and excess water, and the cleaned blood is returned to the patient.

A hemodialysis machine, which pumps blood, adds coagulants, regulates the purification process, and controls the mixing of the dialysis solution and its flow rate through the system. Today, kidney dialysis is used when patients develop complications of the kidney due to liver failure.

Standard treatment may also include dietary protein restriction and a supplement of branched-chain amino acids, together with Bromocriptine, a selective D_2-agonist and Flumazenil, a specific benzodiazepine receptor antagonist. The efficacy of these drugs in hepatic failure and the treatment all in all is questioned and limited, in addition to the fact that many of the patients are refractory to medical management or cannot be treated because of renal failure or other side effects[31, 32].

6.2 Transplantation

Acute transplantation of the liver in suitable cases has shown the most successful survival rates and it is the treatment of choice for liver failure, but this kind of treatment is accompanied with several problems, which will be closer discussed. Nevertheless, patients with acute hepatic failure for instance, have a 50% mortality rate after orthotopic liver transplantation.[33]

One-year survival for liver transplantation in 1960–1970s was 25-35% using methylprednisolone and azathioprine as immunosuppressive agents. However, with the introduction of cyclosporine in the early 1980s, liver transplantation became a clinical reality and now offers one and five-year survivals of 80% and 60% respectively. One-year survival rates of 80-90% are now often expected. The rate-limiting step in the application of transplantation to liver disease has become donor availability[34].

A patient should be considered for liver transplantation when the diagnosis of irreversible end-stage liver disease is made. The most common indications for liver transplantation are chronic liver disease like cholestatic, hepatocellular or vascular disease, and fulminant hepatic failure caused by viruses, drugs or toxins. Other indications for liver transplantation are hepatic malignancies and inherited genetic and metabolic disorders.

The contraindications for liver transplantation can be divided into absolute and relative. The absolute contraindications are sepsis outside the biliary tree, extrahepatic malignancy, advanced cardiopulmonary disease, HIV positivity, active alcohol and/or substance abuse, or the inability to accept the procedure, understand its nature, and cooperate in the medical care required following liver transplantation. The relative contraindications are chronic renal insufficiency, age, and vascular problems, including prior shunt surgery, or other significant extrahepatic disease.

The main beliefs behind the liver transplant preoperative arrangements are to definitively ascertain the etiology of the liver disease and to identify contraindications to surgery. Once the patient is declared a candidate for liver transplantation, the patient goes onto a liver transplant list awaiting a suitable donor, if not the transplant candidate presents with complications that require urgent attention, such as gastrointestinal bleeding or hepatic encephalopathy. Additionally, patients may deteriorate in the course of their disease and become too sick to tolerate transplantation.

Many liver transplant centres expect 15-20% of listed patients to die or be delisted before an organ becomes accessible.

Issues that must be addressed in the postoperative period include monitoring of neurological status, immunosuppression and graft function, management of fluid, electrolytes, and respiratory function. Acute allograft rejection occurs in 40-60% of transplant patients, usually in the first three months after the transplantation[30].

7. Alternative and promising treatment of hepatic failure – liver assist devices in general

7.1 History

The last decades have shown a renewed interest for liver assist devices. However, there have been different approaches through history. The principle difference between mechanical and biological devices is that the mechanical devices are metabolically inert or passive, while the biological are metabolically active. Some of these previously tested approaches will be pointed out in this part.

7.1.1 Different non-biological approaches

There are several mechanical approaches attempting to replace basic liver function. The following approaches will be discussed principally:

- Hemodialysis
- Hemofiltration
- Hemoperfusion
- Exchange transfusion or plasmapheresis
- Albumin dialysis

Hemodialysis: This is already briefly discussed under standard treatment of hepatic failure. The principle is dialysis as in renal substition, ultrafiltrating ammonia and small molecules. The molecular cut-off of the semipermeable membrane is of the same size as for kidney dialysis.

Hemofiltration: This depends on the same mechanism as hemodialysis. This principle use semipermeable membrane with a larger molecular cut-off allowing convective ultrafiltration of larger molecules and toxins across the membrane. This hemofiltration removes toxins by perfusing blood over a bed of adsorbent material, usually a resin compound or charcoal.

Hemodiafiltration: This hybrid method combines diffusion and convective transport. Initially, a special mode of end-stage renal disease treatment, combining the advantages of hemodialysis and hemofiltration, *i.e.* higher elimination rates for smaller and larger molecular weight substances.

Hemoperfusion: Blood, without being separated, is passed over absorbent compounds that bind toxins either by sorption or ion exchange. The blood is perfused through a column of blood compatible charcoal particles.

Exchange Transfusion or Plasmapheresis: This operates on the principle that either whole blood or toxin-bearing plasma is replaced over time to reduce the toxin-content. Two different mechanisms are used to separate blood, either by centrifugation or by membrane filtration. The limitations of this approach are limited by the large amount of plasma needed and the discouraging results.

Albumin dialysis: An approach much alike hemodialysis. Protein-bound toxins in the blood will detach from the albumin and diffuse over a semipermeable membrane with a molecular cut-off below the molecular weight of albumin. The toxins will then again bind to albumin in the albumin circuit. An example of albumin dialysis will be discussed later in the system of MARS.

7.1.2 Different biological models

The following biological treatment attempts for the replacement of the liver have been performed:

- Cross-circulation
- Liver tissue hemo-perfusion
- Organ transplantation
- Heterotopic hepatocyte transplantation
- Bio-artificial replacement

Cross-circulation: This approach links a biocompatible patients circulation with the circulation of a patient with liver failure in a prolonged exchange transfusion that allow the patient's blood to be processed by the healthy individual's liver. However, one of the major side effects of this treatment is adverse reactions to the donor.

Liver tissue hemoperfusion: Similar to the mechanical model of hemoperfusion, except that the absorbent compound is active, *i.e.* there are biocompatible slices of liver. One of the problems with this approach is the oxygenation and nutrition of the liver cells, because of the large size of the slice.

Organ transplantation: The procedure of choice, though severely limited by donor organ supply. Liver transplantation is described earlier.

Heterotopic hepatocyte transplantation: Direct transplantation of hepatocytes suspended in a matrix to provide temporary relief to a failing liver. There is much disagreement on how the transplantation is made with best results, considering location, amount and cell arrangement. One solution is to inject living cells directly into the patient circulation, both the portal vein and the spleen artery has been tested. The cells will adhere to the liver or spleen tissue, and then perform its phenotypic functions.

Bio-artificial replacement: Bio-artificial means in this context biological material, like hepatocytes, combined with mechanical apparatus surrounding the biological material. The bio-artificial device maintains and sustains functional harvested hepatocytes within a matrix that allows for biochemical interactions with patient's blood or plasma. This approach concerning biological LADs is currently applied in clinical trials.

The term alternative treatment of hepatic failure is in this context meaning all other theoretical funded *ex vivo* treatment procedures described above as standard treatment, referred to as a extracorporeal liver assist device, LAD.

7.2 Etiology and liver assist device

The different etiologies of liver disease, cause either acute-on-chronic hepatic failure, depending on whether there existed or not existed previous liver disease. The application of the different liver assist devices, non-biological and biological, have been aimed for different patient groups by the different researchers. Patients with acute-on-chronic hepatic failure have been mainly included in studies with the non-biological devices, while patients with acute hepatic failure have mainly been included in studies with the biological devices. The aim with an LAD is to support the liver in the acute phase and thus make the patient spontaneously recover, or the LAD will act as a bridge to transplantation. The latter application will prevent the patient from further deterioration of the disease, *i.e.* preventing the patients from receiving an even higher Child-Pugh score as before enlisted, and therefore increasing the chance for graft survival. The successful treatment with an LAD, detoxifying the blood, will

prevent the body from eventually developing multi organ failure.

Both patients group have a chance of spontaneous regeneration from the acute phase with LAD treatment, but the prognosis for patients without prior history of liver disease is better. Therefore, the prospects of curing patients completely, is better for the patients with acute-on-chronic hepatic failure. Even though the acute hepatic failure is more challenging to treat, it will save the patients from being transplanted and thus avoiding lifelong immunosuppression.

7.3 The requirements of a LAD

As suggested above, there are two principle needs or functions of an LAD:

- A pre-surgical bridge to liver transplantation
- A bridge to endogenic organ regeneration

The functional requirements of the LAD are to attempt to imitate certain biochemical processes that the native liver routinely performs. In order to conduct these biochemical activities, a LAD will require mechanical functions such as an apheresis unit, a pump for pumping the plasma through the device, and an oxygenator. Thus, the LAD must implement two basic functional requirements:

- Biochemical functions
- Mechanical functions

The liver performs a vast amount of biochemical functions. An optimal device should perform the same functions as a liver. Briefly, the main functions of the liver are the following:

- Detoxification, and drug metabolism (*e.g.* cytochrome p450 activity)
- Metabolic function (conversion of ammonia to urea, bilirubin, cholesterol)
- Synthetic function (albumin, coag. factors)
- Carbohydrate storing and secretion

To mimic all of the above-mentioned functions, a device containing biological material would be able to perform the tasks. For a non-biological device, the vital and important functions must be identified and understood. It is unachievable for any non-biological device to reproduce all of these activities; the total sum of these functions must be narrowed down to essential biochemical functions that will ensure patient survival. Even today, not all of these vital functions are fully understood. Some crucial and vital biochemical functions are recognized:

- Ammonia control - Elevated levels of ammonia found in cerebrospinal fluid of patients with fulminant hepatic failure, and elevated intestinal production of ammonia directly correlate with a progression of hepatic encephalopathy stages.
- Expression and careful regulation of the cytochrome P450 cycle, such as the control of endogenous benzodiazepines, GABA, and the detoxification of acetaminophen.

There are three essential mechanical functions that a LAD must provide:

- Extraction and return of whole blood from the patient to the LAD (an apheresis unit)
- Immune Protection - Isolation of any foreign xenobiological or allobiological components found in the LAD from the body's natural immune response. A physical, filtrating membrane often provides this isolation, which will separate the immune responsive cells and foreign hepatocytes. However, toxins and other liver associated products must be able to move more or less freely over the membrane.

- The nourishment of cells, regarding a biological based unit – providing oxygen and nutrients to the biological component within the LAD.

Historically, a separating line can be drawn among the devices that have been constructed:

- Devices that primarily focus on the mechanical replacement of basic liver function, non-biologically based devices, e.g. hemodialysis.
- Devices that focus on biochemical and global replacement of basic liver function, e.g. biologically based devices.

Though devices are divided along these guidelines, the LADs currently present in clinical trials often use different components from both categories.

7.4 The main components of a LAD

Generally, a LAD is assembled of different parts, see figure 8. Some of the different parts are commercially available, initially intended for other application, *e.g.* plasma separation machine in dialysis treatment. The main parts included into a LAD are:

- An apheresis machine separating plasma from whole blood.
- A pump distributing plasma throughout the circuit.
- A charcoal filter filtering toxins from the plasma.
- In biological systems are a heater and an oxygenation unit included. These prepare plasma to the exposure to liver cells, which need constant heat and oxygen supply.
- In biological systems, a bioreactor or a compartment for the cells of the liver.

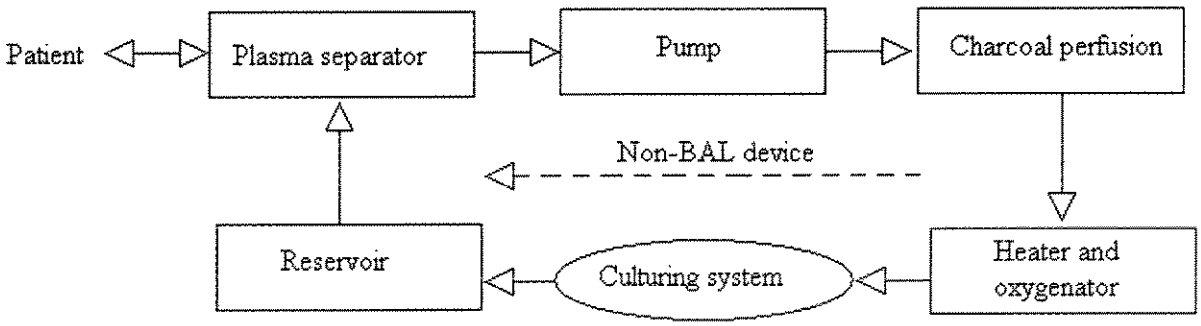


Figure 8: Schematic diagram of the different sections in a liver assist device.

7.5 The principal function of a LAD

The blood is removed from the patient, using catheter with a double lumen catheter inserted into a large vein, for instance the superficial femoral vein. The apheresis machine separates the plasma from the whole blood, returning the cells of the blood to the patient. The pump in the LAD circuit tenderly moves the plasma through the whole system and the potentially harmful toxins due to the hepatic failure are removed by a passive filtering system. This passive filtering system removes potentially harmful toxins and consists of a column loaded with cellulose-coated charcoal.

The liver cells in a biological system, kept in a culturing system of the LAD circuit, are particularly difficult to maintain without an integrated separate heater and an oxygenation unit. The culturing system often consists of a hollow-fibre module in a particular arrangement and may include several billion of liver cells, porcine hepatocytes are widely used. In the culturing system, the plasma will be either exposed

directly to the liver cells, or the plasma will diffuse through a semi-permeable membrane and then exposed to the liver cells. Thus allowing free transfer of materials between the plasma and the liver cells. This is not the case in the non-biological devices where the culturing system is absent. However, a non-biological device can include biological substances in the solution circulating in the LAD circuit, *i.e.* albumin in the MARS unit.

7.6 Two examples of non-biological systems

The following two systems intended for clinical use will be further discussed:

- Molecular Adsorbent Recycling System (MARS)
- BioLogic-DTPF

Different companies have developed these two non-biological systems. MARS is made by Teraklin and BioLogic-DTPF system is made by HemoCleanse Inc. The features of the LADs are illustrated in figures 9 and 10, respectively.

MARS

A commercially available system based on barely biochemical replacement of liver function, is to a certain extent successful in preliminary trials. This system is abbreviated MARS from Molecular Adsorbent Recycling System, based on the principles of extracorporeal albumin dialysis.

The system consists of a hollow fibre dialysis module in which the blood from the patient is dialysed across an albumin-impregnated high-flux polysulfone membrane. A constant flow of albumin-rich dialysate is maintained in the extra capillary compartment, while there is blood in the intracapillary compartment. The albumin-bound toxins from the patient are attracted to the binding sites on the membrane, due to its more adhesivity as it has more free regions for the molecules to attach. Then, the toxins will diffuse to the albumin binding sites in the albumin-dialysate. Albumin, attached to a polymer has a higher affinity for albumin-bound toxins, which is one of the reasons why the toxins detach from albumin in the plasma.

The dialysate is then cleansed by conventional dialysis or filtration to remove the water-soluble toxins. Subsequently, the albumin dialysate is transported over an activated charcoal and thereafter over anion exchange resin column to remove the albumin-bound toxins. Thus the dialysate is regenerated, and the dialysate will be reused in the same circuit. The pore size of the membrane, dividing blood from the albumin dialysate, allows substances with a molecular weight up to 50 kD to move freely. Hence, growth factors, essential hormones bound to carrier proteins, and albumin are not removed from the blood. Smaller toxin molecules that do not adhere to the albumin are removed when they migrate through the minute pores of the membrane into the less-concentrated dialysis solution.

A randomised, controlled clinical study done in 2000 in Germany investigated the efficacy of MARS in treating cirrhosis with hepatorenal syndrome. The study revealed that the albumin-bound substance removal provided by MARS decreased mortality rates. 8 patients out of 13 were treated with MARS together with standard therapy. In contrast, 5 patients in the control group received standard therapy. Between 1 to 10 days, the MARS group underwent one 6-8 hour treatment daily. At 7 days, mortality rates were 100% for the control group and 62.5% for the MARS group. At 30 days, the mortality rate for the MARS group was 70%[35]. However, the sample size is small, and in this perspective is the significance of the trial uncertain.

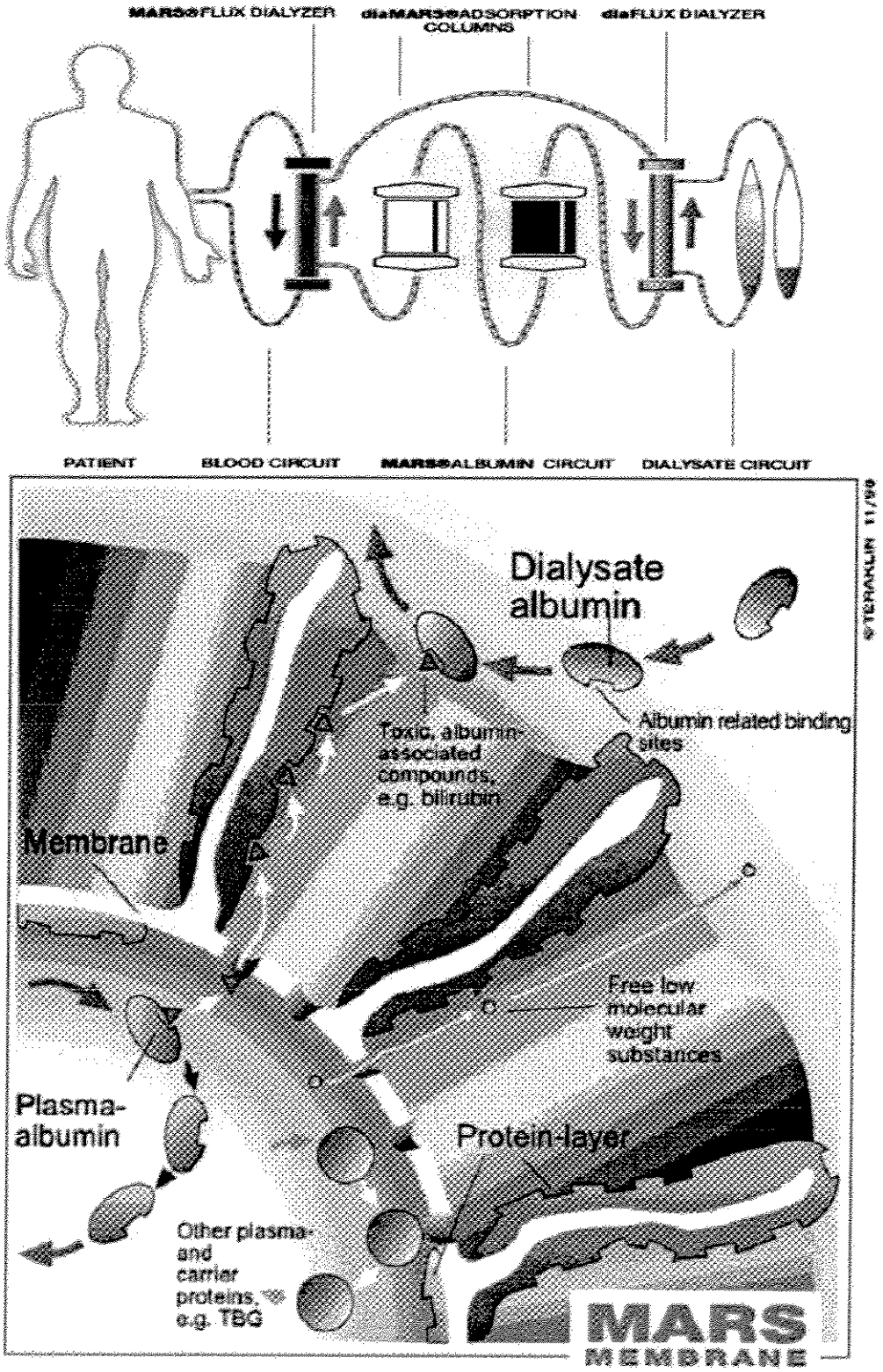


Figure 9: This representation illustrates the circuit and the elimination of toxins in the MARS system. For further description, consult the text.

BioLogic-DTPF

As seen in figure 10, the BioLogic-DTPF combines two parts:

- A module of hemodiabsorption: the BioLogic-DT-system with dialysis against powdered charcoal as sorbent.
- A module of push and pull sorbent based pheresis: the BioLogic-PF-system with powdered sorbent surrounding plasma filters.

Two litres of sorbent suspension surrounds a plate dialysis unit. Changes in pressure in the suspension, generated by the machine, make the membranes pull blood from the patient, and pass it through the dialysis unit. Further, the blood is returned through the same catheter at 200-250 mL/min. The system selectively remove substances with a molecular weight less than 5 kD, such as aromatic amino acids, bile acids, and neural inhibitors like GABA. Urea is removed only moderately. However, the DT-system alone does not remove bilirubin; due to its strong protein binding that prevent the interchange with the sorbent fluid[36, 37]. The suspension is preloaded with glucose due to the hypoglycemia as seen in hepatic failure. The DT-system additionally includes monitors to determine flow rates and thus fluid removal from the patient.

In a randomized, controlled clinical trail, the DT system alone demonstrated a slight improvement in neurologic and physiologic status and an improved outcome for treated patients, compared to non-treated patients with hepatic failure [26]. However, a trial conducted on acute hepatic failure with encephalopathy grade IV revealed no significant improvement in neurologic status or outcome [38, 39].

The BioLogic-PF module consists of a plasma-permeable hollow fibre plasma filter (PF), creating a shifting positive and negative trans-membrane pressure on the returning blood. Further, the pressure causes plasma to transiently pass into a powdered charcoal sorbent suspension around the plasma filter membrane. The intention of the module is to cause an interaction between plasma and charcoal, removing toxins with different degree of binding to a protein like bilirubin and larger molecular weight toxins from plasma. The addition of this module, improves the chemical function of the system.

When the modules are put together, the BioLogic-DTPF system can remove creatinine, and aromatic amino acids at 120-160 mL/min and cytokines at 15-25 mL/min, at a blood flow rate at 200 mL/min. The BioLogic-PF module removes unconjugated bilirubin at 20-40 mL/min. In the trials, the typical treatment protocol with BioLogic-DTPF is 6 hours daily for 3 days and thereafter 2 days of standard therapy [40]. Clinical trials have demonstrated the safety of the system for treatment in acute hepatic failure with encephalopathy, grade III or IV, but the clinical benefit remains to be proven in larger clinical trials [26].

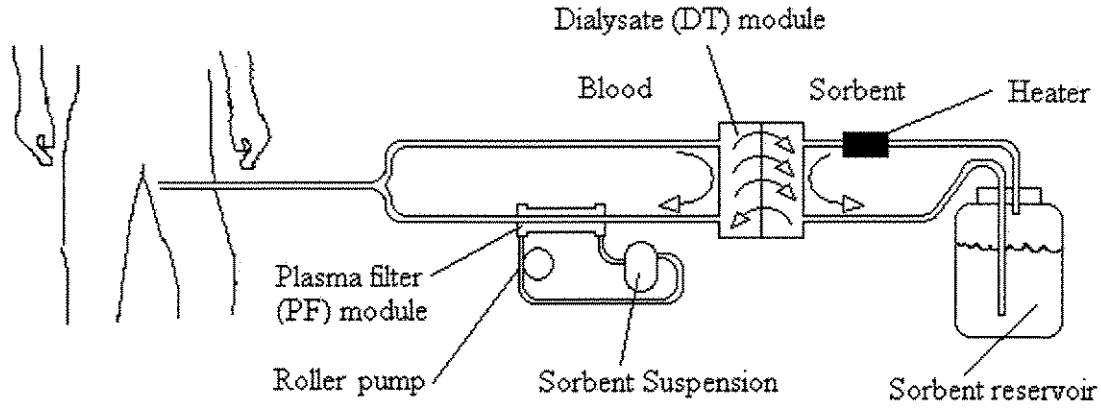


Figure 10: The BioLogic-DTPF system illustrated with its 2 modules: the dialysate (DT) module and the plasma filter (PF) module. Blood is taken from the patient, changed and re-infused through the same canyle. (Ash SR, et al: Push-pull sorbent based pheresis and hemodiabsorption in the treatment of hepatic failure: preliminary results of a clinical trial with the BioLogic-DTPF system. Therapeutic Apheresis 2000; 4; 218-228.)

7.7 The bio-artificial LAD

A bio-artificial LAD combines mechanical and biological principles for a global replacement of primary liver functions. The bio-artificial device maintains and sustains functional, harvested hepatocytes within a matrix that allows for biochemical interactions with patient's blood or plasma. Hence, the components of the bio-artificial LAD can be divided into:

- A mechanical component
- A biological component

The following aspects of the bio-artificial LAD will be discussed:

The biological component of the LAD

- 7.7.1 Human or xenogenic hepatocytes
- 7.7.2 Malignant cells
- 7.7.3 Blood or plasma

The mechanical component of the LAD

- 7.8.1 Culturing system
- 7.8.2 The membrane
- 7.8.3 Distribution

7.7.1 The biological component of the LAD: human or xenogeneic hepatocytes.

The following are the characteristics of the ideal hepatocyte for use in a LAD, as outlined by Jauregui *et al.*:

- Rapidly and easily grown in culture at high densities.
- Non-malignant phenotypes.
- Remain in a well-differentiated state for long period of time.
- Display synthetic and detoxifying features of mature human hepatocytes.

An ideal bioreactor design must support cell attachment, cell-cell interaction, cell-matrix interaction, and scale-up potential.

The usage of either human or porcine hepatocytes has been broadly debated. Human derived hepatocytes are in most cases preferable because they are biochemical compatible to the patient. They assemble equal products that native hepatocytes produce and detoxify and obliterate the similar waste products. Moreover, they are less immunogenic and there is no risk of zoonosis, *e.g.* porcine endogenous retroviruses (PERV).

However, human hepatocytes grow very poorly in culture media. Hepatocytes in an *in vitro* setting such as in suspensions with too high densities of cells, will many cells enter apoptosis or necrotize. Furthermore, there is a problem with accumulating enough human hepatocytes considering the enormous existing liver support burden. Accordingly, hepatocytes from a variety of species have been tested to substitute human hepatocytes. Historically, baboon and porcine hepatocytes are the most successful replacements.

The central problem involving the use of porcine cells in a LAD is their inability to be sterilized. Once hepatocytes are isolated from porcine livers, they cannot undergo any sort of sterilization process and still remain functional. A wide-ranging herd and animal bio-burden screening program has been instituted to provide animals free from zoonotic viruses which could be harvested for different LADs, such as HepatAssist®. With the HepatAssist® there is believed that the semipermeable membrane dividing the plasma from the hepatocytes, further prevents any eventual immunological and zoonotic problems.

Under such screening, the desired porcine cells are cryopreserved and quarantined until the results of the required bio-burden assays performed are acquired. The cryopreservation of the hepatocytes theoretically reduces the risk of immune response, but the risk may still be evident. Once deemed acceptable, the cells remain cryopreserved for easy shipping and storage, and are thawed just prior to the intended usage. Viruses such as zoonotic viruses, including PERV, have not been any problems so far in clinical trials[41].

7.7.2 The biological component of the LAD: malignant cells.

Malignant or immortalised cell lines are used in some LADs. The most commonly used cell line is the human hepatoblastoma cell HEPG2/C3A. This C3A cell line is a clonal derivative of HepG2 that was initially selected for its high albumin production and the ability to grow in glucose-deficient tissue culture medium. Immortalized cell lines provide rapidly proliferating, non-differentiated cells that allow long-term hepatocyte replacement and regeneration. These cells require a minimal seeding considerations and minimal matrix support, to perform its phenotypic functions, as opposed to non-malignant cells. The hepatoblastoma cell line expresses normal liver-specific metabolic pathways such as:

- Ureagenesis
- Gluconeogenesis
- Cytochrome p-450 activity

They also synthesise and secrete clotting factors and other liver-specific proteins as well.

However, they may not respond to physiologic management in the same way as non-malignant cells. There is a question of whether multiple generations will contribute to long-term degradation of metabolic capabilities. It appears that the human HEPG2/C3A cell line have less ammonia clearing capacity as compared to porcine liver cells[42]. In addition, there is a danger of escape from the bioreactor across the semipermeable membrane and subsequently a potential tumour formation. Spontaneous mutations and changes in gene expression during the cultivation are also major concerns.

7.7.3 The biological component of the LAD: blood or plasma.

There are different opinions of what is the most preferable design of the LAD: Should the perfusate be blood or plasma? Blood perfusate has some advantages: It makes the circuit design easy and uncomplicated. Blood include sufficient oxygen and nutrient support for hepatocytes. Nevertheless, coagulation problems appear when blood is used. The use of plasma results in less coagulation problems and it can be filtered before returned to the blood stream. Yet, the circuit is complex and the plasma has

not the same oxygen-carrying capacity of the blood. Many LADs of today perfuse with plasma, using a commercially available plasma separation machine.

7.8.1 The mechanical component of the LAD: culturing system.

At present time, a diversity of bioreactors or bio-artificial livers based on different culturing systems have been developed to perform as bio-artificial livers including:

- Static cultures
- Stirred suspension cultures
- Packed bead reactors
- Matrix cultures
- Fluidised bed cultures
- Hollow-fibre cultures
- Simulated microgravity cultures

Among these various arrangements, cell suspensions, packed bead chambers and hollow-fibre bioreactors have been used clinically. However the treatment of patients with bioreactors currently are based on a limited number of patients. The consequences of the limitation of data are difficulties in telling what significantly approves the condition of the patient and what does not. The hollow-fibre arrangement is the principle design of the device that has gone on to human phase II/III trials, which soon will be completed and evaluated.

Low gravity culture is a culture that is rotating around its axis and generates an environment with reduced gravity forces. It is theorized that the hepatocytes in this manipulated environment are less influenced by shear stress. Therefore, the hepatocytes have better chances to survive and perform its vital and phenotypic functions. This is a way to of diminishing the problems with culturing hepatocytes *ex vivo*.

7.8.2 The mechanical component of the LAD: the membrane

The nature of the membrane separating hepatocytes and plasma can differ in size of the pores and affect the LAD. The differences can generally be divided into:

- Large sized semi-permeable membrane – the large sized pore membrane allows toxin-bearing albumin to cross and interact with hepatocytes. The result is increased overall effectiveness of the LAD, because most of the toxins are coupled with albumin. However, this also increases the immunogenicity of the device, because of higher risk of interaction between hepatocytes and immune cells.
- Small sized semi-permeable - Allows greater immunoisolation, because it prevents toxin-bearing albumin from interacting with hepatocytes and therefore decreases the effectiveness of the LAD.

Hollow-fibre bioreactors, similar to hemodialysis devices, contain a number of hollow fibres of a semi-permeable material. The usage of semi-permeable material allows two compartments, an intra-capillary space (ICS) within the hollow-fibre and an extracapillary space (ECS) outside the hollow-fibre. In most bioreactors in clinical trials today, the hepatocytes are sustained in the ECS and the patient's blood or plasma is perfused through the ICS. The principle of using a semi-permeable material is to let small molecule weight particles diffuse from the blood or plasma in the ICS into the ECS where the hepatocytes are located. At the same time large molecule weight particles remain in the ICS. The molecular cut-off for these semi-permeable materials are around 100-120 kD, while the cut-off for materials used in kidney dialysis is around 10-30 kD.

The ideal hollow-fibre bioreactor will only let toxins and other waste products diffuse across the hollow-fibres and are thus

exposed to hepatocytes. The cells normally found in the blood are not exposed to the ECS environment due to their much larger size. If the cells however become exposed to the ECS environment, two critical problems may evolve, depending on which way the biological material crosses the membrane:

- Cells from the immune system of the host enter the ECS from the ICS and initiate an immune reaction towards the LAD. In worst case, the treatment must be stopped and the patient may die if not transplanted.
- Biological substances from the biological material in the LAD enter the ICS and react with human cells. An example of this is the fear of infection with porcine endogenous retrovirus, PERV, in patients treated with a LAD based on biological content from pigs. However, as already mentioned, some results indicate that this hazard may be low[17].

7.8.3 The mechanical component of the LAD: the distribution.

How can the hepatocytes be evenly distributed and how can this distribution be maintained with time? This question is one of the biggest problems with hollow-fibre bioreactors today and is the subject of a number of investigations.

As already mentioned, within the hepatocytes there exist a gradient of different enzyme activity depending on where the hepatocyte in the sinusoid is located. This causes considerable metabolic consequences. The hepatocytes are also influenced by shear stress. If the shear stress is low, will albumin and urea synthesis dominate, while at high shear stress will cytochrome P450 activity predominate. In conclusion, within the *in vivo* liver each hepatocyte has a unique character and assignment according to its location. An attempt to recreate this multiplicity in a LAD seems very difficult. One way to overcome this problem is to reduce the number of assignments needed to achieve by the hepatocytes.

7.9 Four different biological systems currently being tested clinically

The four following systems intended for clinical use will be discussed:

- HepatAssist
- ELAD
- MELS
- AMC-BAL

Different commercial companies have developed HepatAssist and ELAD, while MELS and AMC-BAL are developed on the authority of two separate universities. See figures 11–15 for the illustrations of each LAD.

HepatAssist

The HepatAssist is meant to function as a bridge to orthotopic liver transplantation or natural liver recovery. The system is an extracorporeal cell-based bio-artificial liver device, based on the use of an open membrane hollow fibre bioreactor. This membrane is microporous, with a pore size of 0.15 μm and a molecular cut-off around 100-120 kD. This size is small enough to halt the passage of cells but large enough to allow for free exchange of soluble and protein-bound toxins and large molecular weight proteins. The cells used in this device are microcarrier-attached primary porcine hepatocytes.

The HepatAssist consists of four parts, much alike the generally described LAD:

- A hollow fibre bioreactor containing primary porcine hepatocytes
- Two separate charcoal filters
- A membrane oxygenation unit
- A pump

The BAL is used in combination with a commercially available plasma separation machine, a heater, and temperature and oxygen monitors. To begin a treatment,

which generally lasts around 6 hours, a patient's blood is separated into blood and plasma in a plasma separation machine. The blood portion is then kept in the plasmapheresis device until reunited with the plasma after processing in the bioreactor.

Once separated from the blood, the plasma, by use of the device's pumping system, is moved via the system through two charcoal filters. These filters essentially act as Kuppfer cells, filtering the plasma from massive bacteria and particulate matter that could overpower the hepatocytes. Thus, they provide the system with its first detoxification. The rest of the detoxification occurs when contact with the hepatocytes are made. After this, the presumably altered plasma is reunited with the stored blood part, and the whole blood is reinfused into the patient.

A membrane oxygenation unit and heater are placed between the charcoal filters and the hepatocyte bioreactor. The heater keeps the plasma at body temperature and since the hepatocytes are in subsequent contact with the plasma, they are kept at constant temperature as well. The membrane oxygenation unit provides the accommodated hepatocytes with the oxygen they require for the appropriate function.

There is some apprehension involving the use of xenogeneic tissue like porcine cells in a LAD as discussed earlier. This is an obvious obstacle with this BAL, especially since the large size of the membrane pores in the HepatAssist does not provide an immunoisolated environment for its porcine hepatocytes. Therefore, great effort has been devoted to the issue of cell supply through the use of intensive animal sourcing programs. The HepatAssist uses cryopreserved hepatocytes, which theoretically reduces the risk of immune response.

In a phase I clinical trial involving three various groups according to their etiology, 18 patients with fulminant hepatic failure 16 patients were bridged successfully to transplantation with the LAD. One patient was bridged to recovery without a transplant, and one patient died because of concomitant severe pancreatitis. Watanabe *et al* who executed the trial experienced encouraging clinical results and a randomised, controlled; prospective phase II-III trial is under evaluation to determine the efficacy of the system[43]. Until the late 2002, 171 patients have so far been analysed.

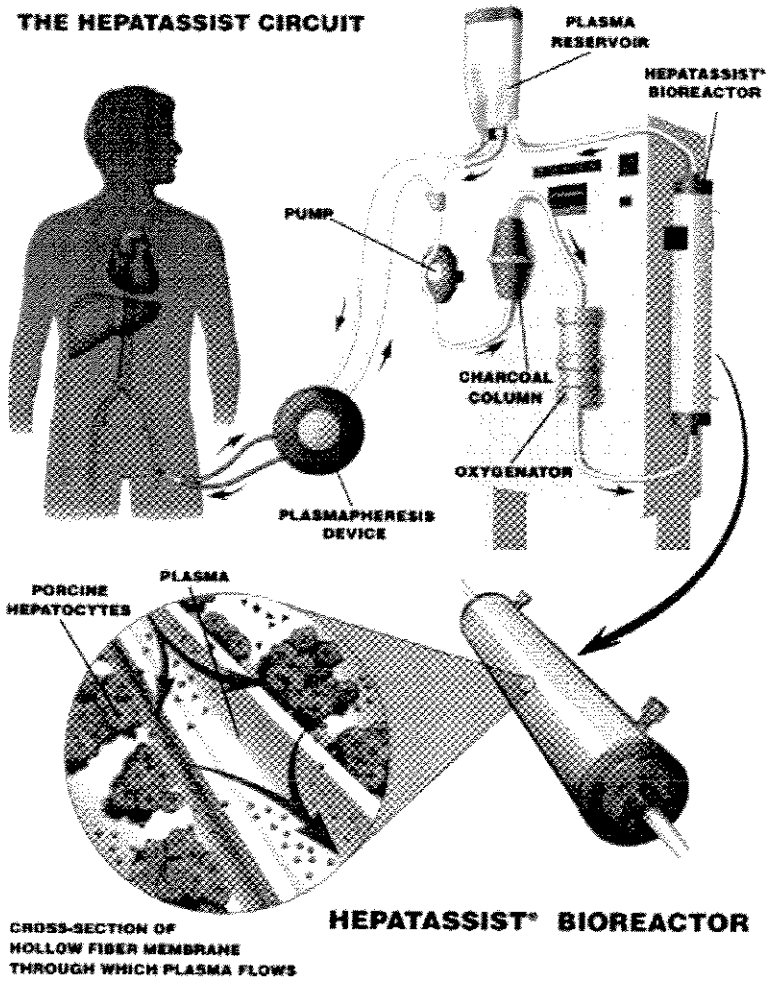


Figure 11: The picture is taken from an advertisement of the HepatAssist with its different parts of the circuit shown. The enlarged section is an illustration of a hollow fibre.

ELAD

In Vitagen's Extracorporeal Liver Assist Device, abbreviated ELAD, is plasma perfused from the patient through the cartridge where it interacts with the metabolically active HepG2/C3A cells. The treated plasma is then filtered and returned to the patient. The basic steps involved in treatment are comparable to those described above in the HepatAssist® device.

The ELAD consists of a hollow fibre cartridge populated with an immortalized liver cell line. The hollow fibre bioreactor contains an attachment surface and small-diameter absorbent fibres that function as a selectively permeable membrane. The genetically engineered cells are cultured into spheroids, which are three-dimensional, tightly packed, freely suspended, and multi-cellular aggregates. The formation of spheroids are believed to enhance the liver specific activity of the cells and thereby detoxifying the toxic compounds of the plasma.

The ELAD cartridge is a disposable single-use device. The cartridge is equivalent to cartridges used in kidney dialysis. The dialysing unit is a two-chambered tube, containing minuscule cylinders. The extracapillary space of the cartridge is inoculated with the human liver cell line. The cartridges are incubated in an automated cell culture, which works to deliver oxygen and nutrients to the cells housed in the cartridges. During a three-

week maturation process, the cells replicate and attach to the outside of the capillaries of the cartridge.

There are four different types of bioreactors that are being used for the ELAD:

- Hollow fibre, which is the most common bioreactor.
- Flat plate.
- Monolayer, perfused beds or scaffolds.
- Beds with encapsulated or suspended cells.

After the cultivation of the spheroids, they are encapsulated in collagen gel. The collagen gel contracts within the lumen inside the bioreactor. This creates an intraluminal space through which a nutrient and hormone rich solution can be circulated as the plasma is filtered through the bioreactor. The hepatocyte spheroids entrapped in collagen gel allow the plasma to filter detoxified compounds through the membrane consequently improving metabolite transport and immunoisolation.

The HepG2/C3A liver cells, located in the extra-capillary space in the gel, can be reproducibly manufactured in culture and express normal liver-specific metabolic pathways such as ureogenesis, gluconeogenesis, and P-450 activity. They also secrete clotting factors and other liver-specific proteins. However, as mentioned earlier, some laboratory analysis indicates altered metabolic capability compared to non-malignant hepatocytes. The metabolic capacity of each cartridge is equal to that of about 200 g of normal liver.

In the late 2002, Millis *et al* used a modified version of the ELAD in a preliminary test where five patients were treated for fulminant hepatic failure until transplantation. Four out of five survived until the 30-day endpoint of the study and the patients tolerated the treatment well. The results indicate that the modified version of the ELAD is ready for larger, randomised phase II/III trials[44]. However, the use of malignant cells call for extra precaution.

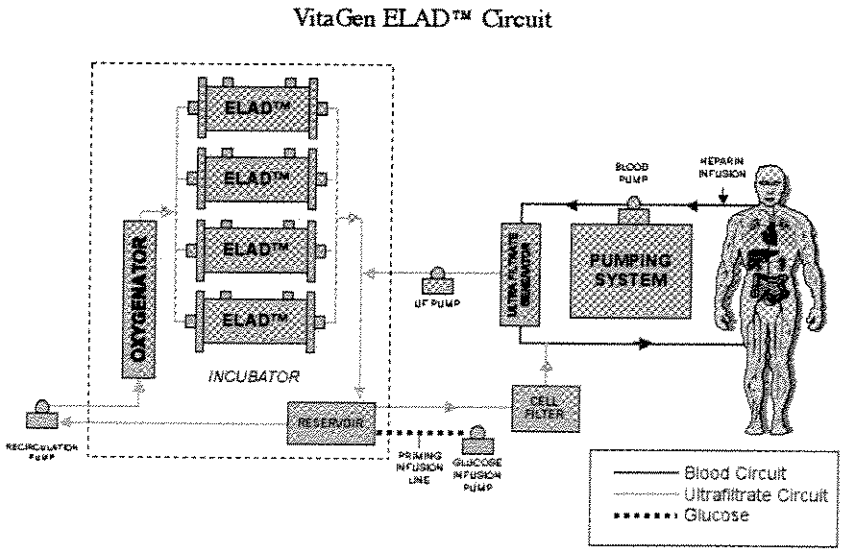


Figure 12: Schematic illustration of the Extracorporeal Liver Assist Device circuit.

MELS

Dr. JC Gerlach et al initiated in the last decade a LAD device entitled MELS, meaning modular extracorporal liver support, at the Charite Institute for Transplantation and Organ replacement, University of Berlin. The LAD uses a three-dimensional design that is different from the

hollow-fibre models that are more commonly used today.

This model essentially involves four different capillary membrane systems, each serving a different purpose, woven into a three-dimensional network. These capillaries independently and locally provide oxygen, nutrients and plasma perfusate inflow and outflow to hepatocytes. This allows for decentralized cell perfusion with low metabolite gradients and decentralized oxygenation and CO_2 removal. Consequently, these has contributed to increased hepatocyte performance in long-term *ex vivo* measurement and the LAD promotes primary human liver cells to spontaneous neo-formation of liver sinusoidal structures *in vitro*. However, its inventive design has also its disadvantage. The major disadvantage is its difficulty to run due to its complexity.

In clinical use, the MELS concept combines different extracorporeal integrating units, modified to match the individual and intra-individual clinical needs of the patient. This include[45]:

- A cell module: This multi-compartment unit contains 400g to 600g of primary human liver cells harvested from human liver donors inappropriate for liver transplant due to cirrhosis, steatosis, fibrosis, or traumatic injury.
- A detoxification module: This unit facilitates single pass albumin dialysis for removal of albumin-bound toxins, reducing the biochemical responsibility of the affected liver and replacing the bile excretion of hepatocytes in the bioreactor.
- A dialysis module: This supplementary unit manages a continuous venovenous hemofiltration, in case of development of hepatorenal syndrome discussed above.

In 2002, the system was successfully used to treat 8 patients with hepatic failure[46] in a preliminary clinical trial. The system is ready for larger, randomised, controlled, prospective trial (phase II-III) to determine the efficacy of the system. However, the material of the efficacy of the system available today is very limited, even compared to the other systems.

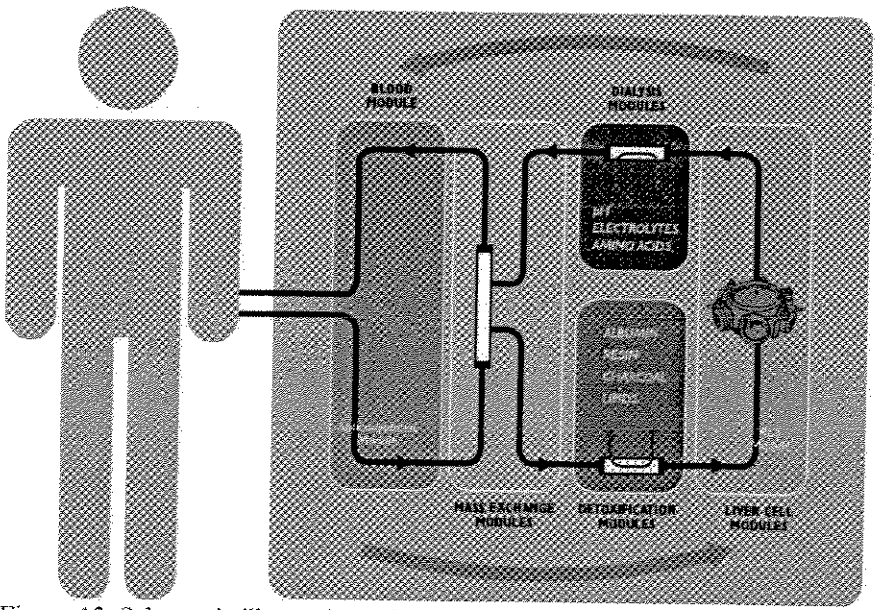


Figure 13: Schematic illustration of the Modular Extracorporal Liver Support circuit, showing the different modules and the circulation within the liver assist device.

AMC-BAL

At the University of Amsterdam, Dr. Chamuleau *et al.* have created and patented an innovative and exciting design for a LAD that may resolve the matrix support and hepatocyte adherence difficulties that so far seem to have outweighed the non-malignant cell based bioreactors. The bio-artificial liver is further designated AMC after its research and development facilities, which are located at the Academic Medical Centre of the University of Amsterdam. In order to adhere the cells more firmly and to preserve their distribution, Dr. Chamuleau has taken a flat hepatocyte-seeded polyethylene sheet and rotated each hollow-fibre one after another to create a tight, spirally wound matrix.

The hollow-fibres provide oxygen from an integral oxygenation unit and nutrient support to the small aggregates of hepatocytes while toxin-bearing plasma is perfused directly through the created extra-fibre space, allowing for direct hepatocyte contact. The primary porcine hepatocytes are attached as small aggregates to the matrix and Chamuleau *et al.* claim that they will function *in vitro* for at least two weeks.

The AMC-BAL has some advantages in compared to other LADs purely based on hollow fibres[47]. In short, these advantages are:

- Oxygenation of the liver cells is optimal because it occurs locally by hollow fibres inside the LAD.
- In the AMC-BAL there is no semi-permeable barrier between the plasma of the patient and the liver cell aggregates, simulating liver cell perfusion conditions like *in vivo*.
- The AMC-BAL can easily contain the equivalent of 200 grams (20 billion of liver cells) or more of liver tissue.
- The AMC-BAL has a simple construction, is cheap, is based on Federal Drug Administration approved materials, and can be steam sterilized.
- Liver cells are attached and function in the AMC-BAL as small aggregates, rather than as single cells.

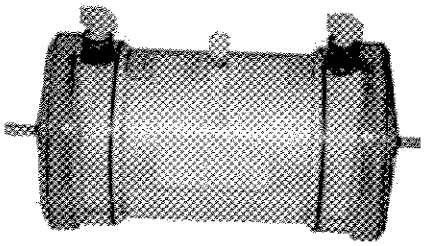
According to the illustrations in figure 14, the system is composed of a dialysis housing (A) comprising a three-dimensional non-woven fabric (B) for high-density hepatocyte culture as small aggregates and hollow-fibre membranes (C) for oxygen supply and CO_2 removal. The combination of the matrix and the oxygenation tubing creates a third compartment (D). These channels are used to perfuse the plasma of the recipient through the bioreactor, which can get in direct contact with the hepatocytes in the fabric. Plasma is perfused through the bio-reactor via the side ports (F). The integrated oxygenator of the bioreactor is connected to the gas supply via the end caps (E). The homogenous distribution of the oxygenation fibres throughout the bioreactor compartment ensures that every hepatocyte has an oxygenation source within its direct surroundings.

The AMC-BAL has been tested extensively *in vitro* and *in vivo* both in small animals and in larger animals. Animals with acute hepatic failure due to surgically induced complete liver ischemia showed significantly prolonged survival time in comparison to control animals. In addition, improvement of several biochemical parameters, for instance plasma ammonia and bilirubin was observed. However, these improvements alone do not provide evidence for a higher survival rate, as considered earlier.

The LAD has been successfully used in a Phase I study in Italy in eight patients with acute hepatic failure. The treatment appeared to be safe without important adverse effects and without transmission of PERV. Seven of the eight patients were successfully bridged to transplantation; one patient recovered during BAL treatment and did not need liver transplantation[48]. The Central Committee on Research Involving Human Subjects does nevertheless; still not

allow the use of the AMC-BAL in the Netherlands because of the theoretical risk of zoonosis.

On this basis, Chamuleau *et al.* initiated industrial research on immortalized human hepatocytes. This research will include the use of genetic modification of foetal human hepatocytes and the use of human stem cells. When these cells are available in sufficient amounts with adequate liver cell functions, then there is also an opening to treat Dutch patients with the AMC-BAL.



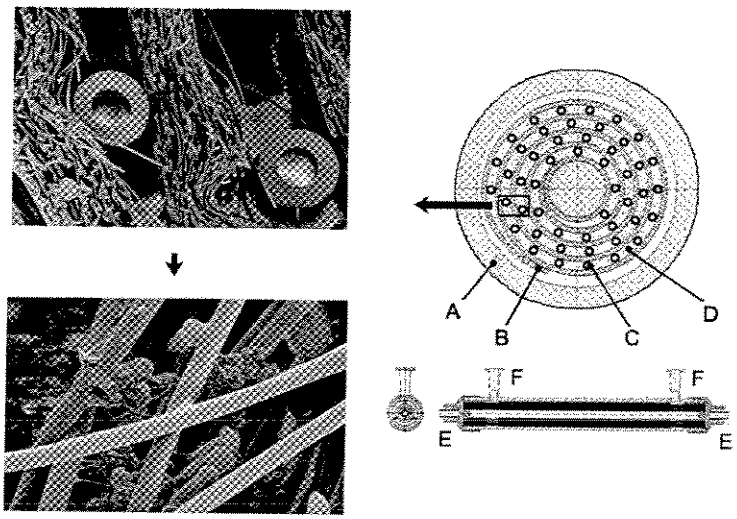


Figure 14: Schematic drawings of transverse and longitudinal cross-sections of the liver assist device (right), a scanning electron micrograph of a small section of the cell culture compartment (middle left), and a scanning electron micrograph of hepatocyte aggregates attached to a scaffold (lower left). The Academic Medical Centre of the University of Amsterdam bioreactor is seen in the upper left.

8. Discussion

8.1 Comparing different devices

This paper has so far looked into six different approaches and some aspects of their advantages and disadvantages. The objective was not to evaluate the different trials. This is, as already mentioned, difficult for several reasons. Mainly, the trials referred to are preliminary and the sample sizes are small. The intention was rather to emphasize the differences, the advantages and disadvantages of each system. Figure 16 is a comparison of the different devices. Today, the major complications associated with the use of liver assist systems are among others increased risk of infections and bleeding. The associated risks emphasize the importance of an overall evaluation of the clinical use of the existing LADs.

When comparing the dissimilar biological liver assist devices in figure 16, some differences are apparent. The phase I/II survival rate to each device is shown. The rate is ranging from 80-100 %. However, all the trials are small sample sized. The inclusion criteria for the MELS and the AMC-BAL are acute hepatic failure. These two have also the highest survival rate of 100%. While the inclusion criteria for the HepatiAssist BAL and the ELAD are more severe liver failure, staged to either III or IV and fulminant hepatic failure, respectively. The survival rates are lower than for the MELS and the AMC-BAL, 89.7% and 80% respectively. One possible explanation is that the patients treated with the HepatAssist BAL and the ELAD had a more severe condition.

As already mentioned, the treatment of choice for liver failure is transplantation. To this point, the different LADs do not present survival rates that exceed the survival rates of standard intensive care[49]. To improve the survival rate, many different extracorporeal methods have been tested clinically. Still many of these systems came short compared to transplantation, indicating the necessity of maintaining the variety of liver functions. The high mortality of certain stages of hepatic failure, and the lack of appropriate treatment is also a reflection of our petite knowledge of the pathophysiology of the disease. Until we know what are the most harmful and fundamental pathological processes that necessitate intervention, will it be challenging to build an extracorporeal device that will prevent hepatic failure from becoming worsened due to the variety of the liver functions. So, many different models for liver supportive systems have been developed based on different hypothesis on the pathological processes responsible for liver failure.

The parameters used in monitoring hepatic failure are numerous, see figure 15. This situation makes the comparability of different studies complex and sometimes impossible. Additionally, the selected outcome of disease also varies, which complicates a potential comparison further. The selected outcome may not be a truthful representation of the progression or regression of the disease. In several experimental studies it is possible to achieve a better score in the monitored parameters, but this does not lead to an improved survival rate compared to conventional treatment of hepatic failure. Again, this is an effect of our lack of knowledge of the pathophysiology of hepatic failure.

Hemodynamics
Intracranial pressure, ICP
Cerebral perfusion pressure, CPP
Gastric pH
Serum electrolytes, *e.g.* K^+
Metabolic state, *e.g.* glucose level
Respiration
Coagulation state
Consciousness
Urinary output
Liver function tests, *e.g.* ALAT
Blood urea nitrogen
Creatinine level
Complete blood cell count

Figure 15: Examples of parameters used in monitoring hepatic failure.

Though sources differ on exactly how much of the liver's bio-mass must be replaced to provide a minimum amount of liver function, Cuervas-Mons *et al* developed an experimental porcine model of hepatic failure, treated pigs with a bio-artificial liver containing 6 $x10^8$ porcine hepatocytes or 3-5 % of the liver mass and improved significantly survival, indicating the minimum required cell mass[50]. As seen in figure 16, the bio-artificial LADs mentioned here contain at least this volume. For instance, if a patient's liver weighs 1.5 kg, the 100 g in the ELAD represents approximately 7%.

Clinical improvements have also been seen in hepatocyte transplantation studies using less than 10% of the host's liver mass. Thus, a LAD must be created and tested to

provide temporary relief of liver function, while the native liver undergoes the hepatocyte regenerative process. Consequently, more livers will be available for transplantation on other clinical indications, and costly transplant procedures can be avoided.

More important for the patient, the need for life long use of immunosuppressive agents such as FK506, Neoral, Cyclosporine, CellCept and Imuran after transplantation will no longer be required. This type of liver-assist is most clearly indicated when the liver of patients has been considerable resected, like resection for cancer, or for liver trauma.

ELAD

The main reason for hesitation regarding the ELAD is the choice of the cellular component, the malignant HepG2/C3A cells. The risk of transmitting oncogenic substances or cells into the circulation of the patient is present, and filters may be included in the ELAD devices as an extra precaution. Additionally, as mentioned the malignant cells have different biochemical characteristics compared to primary cells. This may affect the efficacy of the system. Although the safety issues have been settled in the ELAD, there have been no uniform standards for testing the efficacy due to the small sample sizes in the trials. Nevertheless, various ELADs are gaining attention as promising treatments for liver failure.

To improve the LAD further, a better understanding between liver regeneration and the bio-artificial devices will be crucial in optimising the effectiveness of the ELAD. Ideally, the bioreactor designs should support cell attachment, cell-cell interaction, cell-matrix interaction, and scale-up potential. The support of polarity and subsequent different characteristics of the hepatocytes due to localization in the ELAD by using malignant cells is questionable. It is possible that the cells are the reason for its promising results, but it may at the same time also prevent them to polarize and organize as an *in vivo* organ. Co-culture of immortalized human liver cell lines with non-parenchymal liver cells has been shown to be beneficial. Although, the addition of other cell types would make the design, the construction, and the management of the LAD even more complicated. Another shortcoming of the LAD is its long preparation time of three weeks. By this time many patients with severe hepatic failure may deteriorate or die. In this aspect it is still a model for experimental use only.

HepatAssist

The most favourable aspect of this device is by large its positive trials and considerably tested functions. As mentioned, it is currently in a phase II/III trial and 171 patients have been included up until late 2002. Hopefully, it will soon be ready for use outside an experimental setting. On the other hand, the LAD is colossal and requires excessive expertise to operate it. Additionally, the device is in some degree outdated by newer design, which bypass the disadvantages with the system. It is difficult to tell whether it is the complete or just parts of the system that provide the efficacy of the system, since the HepatAssist is composed of charcoal filters and a hepatocyte bioreactor. The system uses cryopreserved porcine hepatocytes, which include a theoretical risk of zoonosis due to viruses.

MELS

An important negative aspect of the MELS system is its difficulties to run because of its intricate three-dimensional network. As with the ELAD, the device has long preparation time that is disadvantageous for the patient. Since the system is quite new, its true efficacy and potential is still unknown. The trials so far are of small sample size, in fact the smallest size of all the biological based systems, and may only indicate the safety of system without the important adverse effects. However, the material of the system available today is very limited. The addition of a dialysis module for renal support is making the system more complete for a critical ill patient with several affected organs, *i.e.* hepatorenal syndrome. Yet, there

is a difficulty in telling whether the addition affects the efficacy of the system, and thus making the MELS appear more efficient than it truly is.

AMC

The AMC-BAL has a new, promising design, which bypasses several problems with the semi-permeable hollow-fibres, and the system seems to maintain the polarity much alike parenchymal hepatocytes. However, the design, without a filter between the plasma and the cellular component, also involves a higher risk of immune response due to greater interactions between the BAL and the immune system of the patient. In addition, the device does not have long preparation time, as many patients have an urgent need for treatment. The AMC-BAL has a simpler construction, it is cheap, it is based on Federal Drug Administration approved materials, and can be steam sterilized. Since the AMC-BAL is fairly novel, its efficacy is still unknown and the initial trials so far only point to the safety of the system, *i.e.* screening for harmful agents.

8.2 The biologic LADs versus the non-biologic LADs

Different aspects of both the biologic and non-biologic LADs have now been assessed. When comparing these different approaches, some differences are obvious. As mentioned, the biologic LADs have, at least in theory, some metabolic and homeostatic functions like the synthesis of essential serum proteins, excretion of bile and other related products and regulation of nutrients.

The metabolic and homeostatic functions are not present in the non-biologic systems; they can only detoxify substances in the blood. In contrast, the major advantage of the non-biologic systems is their independence of immune protection, in contrast to biological systems. Additionally, the systems do not need to provide a viable environment for living cells. The practical consequence is a more simple and straightforward system to build and to maintain, but with substantial limitations regarding functions.

The non-biologic systems have only proven a slightly reduced mortality rate compared to standard treatment. The biological systems have shown the same efficacy as standard treatment, and are therefore clinically inferior. However, there is a major difference between the biologic and the non-biological LAD trials: this discrepancy is found in the selection of patients. The intention is to reverse the progression of the failure, by replacing the native liver functions. The non-biological LAD trials have included patients with acute-on-chronic liver failure.

On the other hand, as is the case for biologic LAD systems, the patients included are those with acute hepatic failure. This diagnosis is much more severe, and the outcome is rather poor, and the search for effective therapy is more impulsive and consequently involve higher risks for the patients. In acute hepatic failure regeneration of new hepatocytes is likely to occur, so the LAD will act as a pre-surgical bridge while waiting for the availability of an appropriate transplant.

To recapitulate, the non-biological are simple and straightforward to build and to maintain, but their future potential is rather poor due to their limited functions. According to the trials their efficacy is better than for the biological system, but this is likely due to selection of the patients. The patients included in the former have acute-on-chronic liver failure, while the inclusion criteria for the latter are acute liver failure. On the other hand, the biological systems may have a larger future potential but then again they have a more complex design.

8.3 The future

Generally, the history of the development of the LAD has been intriguing, exhibiting the typical slow-growth curve from 1950-1970 and the subsequent rapid acceleration due to the initiation and incorporation of the bio-

hybrid knowledge. An excess of liver support devices have reached varying stages of conceptualisation, manufacturing and experimental implementation. Though research interests in the bio-artificial liver are constantly growing, the devices that have been produced have so far had marginal success.

There are several obstacles before reaching a well-functioning LAD. Firstly, despite decades of research, there are still difficulties of prolonging the viability and the performance of the hepatocytes *ex vivo*. Moreover, hepatocytes are more challenging to adhere to a surface or matrix, complicating the incorporation of cells and synthetic materials into a LAD. Today, the solution is malignant or xenogenic cells, but this addresses major safety issues. The use of genetic modificated human hepatocytes and human stem cells are encouraging, since this will replace the use of malignant and xenogenic cells.

In the future, a further challenge is to arrange the hepatocytes within the LAD successfully, so they polarize, organize and differentiate in the same way as a complete, well-functioning organ with a variety of functions. This includes the production of bile. The LADs of today are far from produce bile separately from other features. To be able to evolve a well-functioning LAD, such as in co-culture with other non-parenchymal liver cells, it is important to unravel the basic processes of the pathophysiology of the hepatic failure, including the production of bile. For instance, co-culture of immortalized human liver cell lines with non-parenchymal liver cells has been shown to be beneficial. If the right parameters are set, including growth- and differentiation-factors, the ideal BAL may evolve to function as a liver *ex vivo*.

The distinctive design of the bio-artificial LAD of today includes a column with hollow-fibre capillaries containing plasma. Hepatocytes are located in the extracapillary space alone or are attached to a matrix such as microcarriers. However, the continuation of hollow-fibres in the future is questionable. The use of hollow fibres in a LAD is perhaps evolving to be more than just a barrier between the cellular component and the plasma of a patient, *e.g.* the AMC-BAL is an example of revolutionary use of the fibres.

Consequently, the need for basic research to reveal the biochemical control of growth and regulation is evident, since the results of today are what they are. In the future, a LAD will need to be much more to the point, fulfilling precisely the failing functions of the liver of the host. To get there, numerous large scale, prospective, and randomized trials with comparable end-points to evaluate the different approaches are important for further progression. As a start, research on the fundamental functions of the cells of the liver and the pathophysiology of the hepatic failure is needed.

Figure 16: A comparison of different liver assist devices (I / II)

	Name of device	Shortening	Developed by	Cellular component	Anticoagulation	Inclution criteria
1.	BioLogic DTPF (HemoCleanse)	Detoxification, Plasma Filtre	Ash et al.	None	None if minimal coagulopathy	Fulminant hepatic
2.	MARS (Teraklin)	Molecular Adsorbent Recycling System	Stange, Mitzner et al.	None	Heparin or sodium citrate	Cirrhosis with hepatorenal syndrome
3.	ELAD (Hepatix/Vitagen)	Extracorporeal Liver Assist Device	Sussman, Jauregui et al.	C3A cell line	Regional heparinization	Fulminant hepatic failure
4.	HepatAssist BAL (Circe Biomedical)	Bio-Artificial Liver	Demetriou et al.	Porcine hepatocyte	Sodium citrate	Acute liver failure, stage III-IV
5.	MELS (Charite Institiute)	Modular Extracorporeal Liver support	Gerlach, Sauer et al.	Human liver cells	Heparin	Acute liver failure
6.	AMC-BAL (University of Amsterdam)	Academic Medical Centre Bio-Artificial Liver	Chamuleau et al.	Porcine hepatocyte		Acute liver failure

Figure 16: A comparison of different liver assist devices (II / II)

Name of device	Blood flow rate (ml/min)	Cell amount	Duration in hours	M.W. Cutoff	Phase I/II survival rate*
1. BioLogic DTPF (HemoCleanse)	200-250	None	6	5000	Not available
2. MARS (Teraklin)	100-250	None	2	50 000	70 %
3. ELAD (Hepatix/Vitagen)	150	~100 g	120	120 000	80 %
4. HepatAssist BAL (Circe Biomedical)	90-100	5×10^9 cells	8	120 000	89.7 %
5. MELS (Charite Institiute)	300	400-600 g	500	400 000	100 %
6. AMC-BAL (University of Amsterdam)	30-150	200 g	24	Cellular	100 %

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