

The promise of artificial liver replacement

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1. ABSTRACT

One of the most challenging clinical syndromes in medicine is that of acute liver failure (ALF). Many devices and systems have been devised to support ALF patients. This manuscript reviews the significant clinical findings of ALF, as well as, the non-biologic liver support systems and the bioartificial liver devices that have been clinically tested to support patients with this disease. Finally, we identify several improvements critical to the future of the field of bioartificial liver replacement therapy.

2. INTRODUCTION

Throughout the 1900's advances in medical technology have provided clinicians with the tools to support acutely failing organ systems. In the setting of acute respiratory failure, patients are regularly intubated and supported with mechanical ventilation. Neonates born before their lungs are completely developed are supported with Extra-Corporeal Membrane Oxygenation (ECMO) until their pulmonary system gains competence. Patients suffering from acute cardiogenic shock are regularly

Table 1. Selected serum proteins produced by the liver

Protein	Function	Molecular Weight (kDa)
Albumin	Binding protein, osmotic regulator	66.5
Anti-Thrombin III	Thrombin Inhibitor	65.0
Complement C1	Complement Pathway Component	86.0
Complement C2	Complement Pathway Component	117
Complement C3	Complement pathway component	185
Complement C4	Complement pathway component	200
Complement C5	Complement Pathway Component	180
Complement C6	Complement Pathway Component	95.0
Complement C7	Complement Pathway Component	100
Complement C8	Complement Pathway Component	153
Complement C9	Complement Pathway Component	79.0
C-reactive protein	Binds pathogens and damaged cells to initiate their elimination	118
Factor II	Pro-enzyme of Thrombin	72.0
Factor V	Cleaves Prothrombin to Thrombin	330
Factor VII	Extrinsic Coagulation Pathway - Factor X activator	50
Factor X	Initial Component of Common Coagulation Pathway	56
Factor XIII	Fibrin Cross-Linking	340
Ferritin	Intracellular iron storage	450
Fibrinogen	Precursor to fibrin in hemostasis, wound healing	340
Haptoglobin	Binds hemoglobin released by hemolysis	100
Transferrin	Iron-binding protein	79.5
Plasminogen	Proenzyme of Plasmin	81.0
α_1 -antitrypsin	Inhibitor of elastin degradation	54.0

Adapted from references 5, 142

supported with intra-aortic balloon pumps; Left Ventricle Assist Devices (LVAD); and pacemakers. Acute Renal Failure is commonly treated in the Intensive Care Unit setting with Continuous Veno-Venous Hemofiltration and Dialysis (CVVHD). Even Total Parenteral Nutrition (TPN) can be administered for patients with acutely decompensated alimentary tract function.

Perhaps the most widely applied technique for support of a chronically failing organ system is the use of hemodialysis for renal failure. As early as 1913, researchers at Johns Hopkins Hospital performed successful hemodialysis on rabbits and dogs using an artificial kidney made of collodion. This technology was later applied for the first time in humans in a 1925 German study conducted by G. Haas. Over the following three decades, the work of W. Kolff (1) and the development of new membrane technologies lead to the first commercially available dialyzing machine – the Baxter/Travenol recirculating U-200 twin coil dialyzer. By 1960, Scribner *et al* described the treatment of chronic uremia using intermittent hemodialysis. By 1972, hemodialysis was so widely accepted as an effective treatment for chronic renal failure that Congress passed the End-Stage Renal disease act. This act ensured federal support for chronic kidney disease management (2).

The goal of all of these treatment modalities is to support the patient until their organs: a) recover through the natural course of their disease or b) are replaced through organ transplantation. However, despite the success of all the current organ support systems, the management and support of a failing liver in both the acute and chronic setting has remained a significant clinical challenge.

2.1. The normal liver

The human liver is the largest internal organ of the body and is its central metabolic factory, performing life sustaining functions of synthesis, storage, secretion,

regulation, detoxification, and excretion (3). The liver synthesizes approximately 90% of the proteins in blood plasma and about 15% of the total protein mass of the body. Essential functions of the liver include secretion of plasma proteins, gluconeogenesis, glycogen storage, glucose metabolism, cholesterol homeostasis, bile salt production, and detoxification of endogenous metabolites and exogenous substances. The plasma proteins secreted by the liver perform a wide range of functions ranging from hemostasis to the maintenance of normal plasma osmotic gradient. The select group of proteins presented in Table 1 emphasizes the liver's synthetic role in the process of hemostasis (4, 5).

Glucose is the primary energy source for the brain and renal cortex and the liver plays a central role in glucose metabolism (6). Through its ability to store and release systemic nutrients, the liver can continuously provide adequate blood glucose levels for normal nervous and renal system function during varying states of absorption and starvation. The liver also serves as the primary site for processing fatty acids and cholesterol from the diet and peripheral tissues, packaging them into lipoprotein complexes, and releasing the complexes into the circulation (6). A detailed description of these metabolic processes is beyond the scope of this text, however it is easy to appreciate from this cursory discussion that the loss of a fraction of the liver's function could have grave consequences.

Aside from its homeostatic roles, the liver is also responsible for detoxification and elimination of drug and environmental toxins. Of specific interest within the context of bioartificial liver replacement is the liver's metabolism of ammonia. Ammonia is a key intermediate in nitrogen and protein metabolism, and is primarily produced in the colon. Ingested proteins and secreted urea are degraded by bacteria with liberation of ammonia, which is then absorbed into the portal circulation. Typically the liver

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clears these toxins with great efficiency preventing their entry into the systemic circulation (7). In a healthy liver hepatocytes rapidly convert ammonia into glutamine and ultimately into urea for secretion by the kidneys (8). This process exemplifies the liver role as the filter for toxins introduced into the body through the GI tract.

2.2. Biochemical evaluation of hepatic function

With so many varied roles, biochemical assessment of the liver involves careful interpretation of abnormalities in the context of a carefully obtained history and thorough physical examination. The significance and interpretation of these laboratory studies is also beyond the scope of this text. The following represents a summary of the tests most commonly used as indicators of the severity of liver failure and as benchmarks to evaluate the performance of bioartificial liver replacement devices (9).

2.2.1. Markers of synthetic function

The prothrombin time (PT) is a laboratory measurement of the conversion rate of prothrombin to thrombin and provides an estimate of hepatic synthetic function because it depends on the activity of several clotting factors synthesized by the liver including factors II, V, VII, and X (9). Since PT values can vary from lab to lab, the international normalized ratio (INR), was developed as a standardized method to monitor anticoagulation therapy and is also used to evaluate the progress of patients with acute liver failure (10).

The liver synthesizes approximately 10 g of albumin per day. Many factors diminish the efficiency of albumin synthesis including malabsorption, malnutrition, renal disease, and systemic hormonal deficiencies. Therefore, serum albumin levels are not a specific indicator of liver function. In addition, the serum half-life of albumin is 21 days making serum albumin concentration a poor marker of acute hepatic synthetic dysfunction (11). However, the serum albumin level can be used as a prognostic indicator in patients with chronic liver disease and is frequently used in the *in vitro* evaluation of bioartificial liver (9).

2.2.2. Markers of excretory function

Bilirubin is a product of hemoglobin catabolism and normal serum bilirubin levels are less than 1 mg/dL (17.1 $\mu\text{mol/L}$). Two types of serum bilirubin are evaluated biochemically: direct and indirect. Direct bilirubin is a water-soluble, conjugated form of bilirubin, and indirect bilirubin is a lipid-soluble, unconjugated form. Conjugated, or direct, bilirubin makes up a small fraction of the total normal serum bilirubin and is typically an indicator of hepatobiliary disease. Excretion of bilirubin by the hepatocyte is the rate-limiting step in bilirubin metabolism and defects in the hepatic excretion of bilirubin result in secretion of conjugated bilirubin from hepatocytes into the serum (9). Typically unconjugated, or indirect, serum bilirubin reflect a dynamic equilibrium between the rates of bilirubin production and hepatobiliary excretion and composes more than 90% of total serum bilirubin in a normal adult (12). Hemolysis, hematoma resorption, and muscle injury can increase production of bilirubin and

subsequently increase the serum unconjugated bilirubin level with minor clinical significance (9). The conjugated serum bilirubin level is an inversely proportional indicator of prognosis in patients with acute-on-chronic liver failure and is used in the formula to rank the acuity of patients with end-stage liver disease who are awaiting liver transplantation (13, 14).

2.2.3. Markers of hepatocellular damage

The aminotransferases, aspartate aminotransferase (AST), and alanine aminotransferase (ALT), catalyze the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to form oxaloacetic acid and pyruvic acid, respectively, during gluconeogenesis (9). ALT is localized primarily in the liver cytoplasm, whereas AST can be isolated from the cytoplasm and mitochondria from a spectrum of tissues including liver, cardiac and skeletal muscle, kidney, brain, pancreas and blood cells. Elevated serum aminotransferase levels indicate hepatocyte injury or necrosis (15). The leakage of aminotransferases from hepatocytes can be triggered by various types of liver diseases, including viral hepatitis, ischemic injury, and toxin- or drug-induced hepatotoxicity. Changes in serum aminotransferase elevations do not correlate directly with the severity of hepatocellular necrosis and serum aminotransferase levels can be within the normal range in patients with advanced fibrosis or cirrhosis (16). Two common causes of significant aminotransferase elevations are acute viral hepatitis, and acetaminophen overdose. Both disorders can result in extreme increases in aminotransferase levels ($> 1000 \text{ U/L}$); however, the characteristic serum AST-to-ALT (AST/ALT) ratio of less than 1 remains unchanged (17). In patients with hepatitis, sudden decline in aminotransferase levels associated with increasing serum bilirubin level and prolongation of the prothrombin time indicates the development of acute liver failure (9).

2.3. Liver failure

In the United States, approximately 30 million people suffer from liver disease, which is about 1 in 12 Americans. Each year over 43,000 people die from liver disease with an annual cost of over eight billion dollars (18). These patients experience a progressive worsening of their liver function and many eventually progress to acute-on-chronic liver failure. In addition to patients with chronic liver disease, acute liver failure can develop in healthy patients from such causes as viral hepatitis and acetaminophen-toxicity (19, 20). Broadly defined, acute liver failure (ALF) is a clinical syndrome of coagulopathy and mental status changes, or encephalopathy, evidenced within 8 weeks of the sudden loss of hepatic parenchymal and metabolic function. ALF represents the final common pathway of severe liver injury, and constitutes a medical emergency associated with the development of cerebral edema, bleeding, and infectious complications (21). While other definitions of ALF have been introduced in the literature in an effort to improve prognostic accuracy, the original 8 week definition is the most widely used in clinical studies and in criteria for liver transplantation in the United States (22).

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The clinical features of ALF are manifestations of dysregulation of normal metabolism; the loss of protein synthesis; and loss of metabolic detoxification as a direct result of hepatocellular injury (20). Patients typically present with nonspecific complaints such as nausea, vomiting, and malaise, however as the disease progresses more significant derangements become evident (22). The dysregulation of normal metabolism, including a failure of gluconeogenesis; glycogenolysis; and lactate processing, rapidly results in hypoglycemia and acidosis leading to acute mental status changes and hypotension (23, 24). Coagulopathy develops as the loss of protein synthesis results in decreased plasma levels of coagulation factors I, II, V, VII, IX, and X increasing the risk of gastrointestinal and intracranial hemorrhage. Finally, the loss of metabolic detoxification results in the accumulation of nitrogenous metabolites which leads to the development of hepatic encephalopathy. In addition to hypoglycemia, coagulopathy, and hepatic encephalopathy, patients with ALF are also at substantial risk for infections, Acute Respiratory Distress Syndrome (ARDS), and renal failure (22-24). These clinical features and their management are the subject of many excellent reviews (21, 23, 24) and are summarized nicely by Fontana in Sleisenger & Fordtran's *Gastrointestinal and Liver Disease* 8th edition (22). Coagulopathy and hepatic encephalopathy will be discussed in further detail here as they relate directly to the efforts of many researchers in the field of bioartificial liver replacement.

2.3.1. Coagulopathy

Patients with acute liver failure develop coagulopathy with an increased risk of major hemorrhage and Disseminated Intravascular Coagulation (DIC). This complicated coagulopathic syndrome develops because the liver is the primary organ responsible of the constitutive production of pro-coagulation factors including coagulation factors I, II, V, VII, IX, and X and anti-coagulation factors including plasminogen and anti-thrombin III (8). The liver is also involved in the clearance of activated clotting factors and their degradation products (25). Of the coagulation factors synthesized by the liver, factor V has the shortest half-life and can be serially monitored with PT in patients with acute liver failure to assess the severity of coagulopathy due to loss of hepatic synthetic function (22).

Approximately 1 in 5 patients will develop clinically significant bleeding during the course of ALF (25). The most disastrous bleeding events are intracranial and gastrointestinal. Intracranial bleeding is frequently a late, albeit, terminal event in these patients since it is usually preceded by the development of cerebral edema and intracranial hypertension (23). Gastrointestinal bleeding frequently develops earlier in the course of ALF as a result of pre-existing portal hypertension and is typically located in the upper GI Tract (22). As large volumes of blood enter the lumen of the GI tract and are broken-down, the failing liver is presented an enormous load of ammonia and other nitrogenous metabolites it is incapable of clearing. This leads to the development of another catastrophic consequence of ALF: Hepatic encephalopathy (7).

2.3.2. Hepatic encephalopathy and cerebral edema

Hepatic encephalopathy is a defining criterion for acute liver failure; however the precise pathogenic mechanisms are not fully defined. The presence of ammonia in the systemic circulation is frequently implicated however no clear mechanism explains how ammonia produces mental status changes (23). The failure of the liver to clear the nitrogenous by-products of protein breakdown absorbed from the colon appears to play a key factor in the development of hepatic encephalopathy (26). In the setting of ALF coagulopathy, gastrointestinal bleeding is common and the breakdown of blood in the intestine liberates ammonia and other neurotoxins (7). However, in ALF multiple metabolic abnormalities coexist, including changes in the profile of circulating amino acids, mercaptans, and central nervous system levels of dopamine and other neurotransmitters (27). When the liver fails to clear these substances encephalopathy ensues (8).

Cerebral edema is found in up to 80% of patients who die of acute liver failure and is nearly universal among patients with coma. Increased production of glutamine in the central nervous system as a result of high circulating levels of ammonia and intracerebral lactate are believed to be critical to the pathogenesis of cerebral edema (28). Progressive cerebral edema associated with the development of intracranial hypertension also can result in cerebral hypoperfusion and consequent cerebral hypoxia that can lead to irreversible neurologic damage, uncal herniation, and brain death (29). Loss of intracranial vascular tone can lead to surges in intracranial pressure (ICP), with changes in systemic pressure. In addition to cerebral edema, many of the complications of acute liver failure, including hypoglycemia, sepsis, fever, hypoxemia, and hypotension, may contribute to neurologic abnormalities (22).

The Working Party of the 11th World Congress of Gastroenterology defined hepatic encephalopathy in four stages (30). Stage 1 is characterized by subtle changes in affect, euphoria, anxiety or difficulties with concentration. Stage 2 is characterized by drowsiness, apathy, and mild disorientation. Patients in stage 3 demonstrate marked somnolence with response to verbal stimuli, confusion, gross disorientation and incoherence. Stage 4 is defined as frank coma with no response to noxious stimuli detected. Asterixis and tremors are common features in stage 1 or 2 encephalopathy and hyperreflexia, clonus, and muscular rigidity are frequently seen in stages 3 and 4. All of these clinical features may be fully reversible with treatment however; encephalopathy of this degree is typically associated with a poor long-term prognosis (8).

Although the precise mechanisms are not established, ammonia is a clinically useful marker of the production of enteric toxins from nitrogenous substrates (7). Blood ammonia levels may be measured when hepatic encephalopathy is suspected, both for diagnosis and as a guide to treatment. Elevated blood ammonia levels are detected in 60% to 80% of patients with cirrhosis and encephalopathy, however normal ammonia values do not exclude the diagnosis (22). Many well-conducted studies

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have demonstrated that reduction of blood ammonia concentration is associated with resolution of hepatic encephalopathy (31-42). Therefore, the primary goal of treatment for hepatic encephalopathy historically has targeted various mechanisms of ammonia clearance (8).

2.4. Current treatment for liver failure

Currently, the only effective treatment for patients with chronic liver disease is liver transplantation. Liver transplantation became accepted as Standard of Care in 1983 after a National Institutes of Health Consensus Conference deemed it an effective therapy for liver disease (43). Today, 17,500 Americans await a liver donation; however, due to a severe organ donor shortage only 6,500 liver transplants are performed each year in the United States. In 2005, 2,104 patients died while waiting for a suitable liver for transplantation (44). Despite advances in medical management ranging from medical therapies such as glucocorticoid therapy and administration of prostaglandins to extracorporeal support systems, mortality rates in patients with liver failure remain high, approaching 80% in the absence of liver transplantation (45). Unfortunately, many patients with irreversible acute liver failure do not undergo transplantation, because of late referral, or the lack of a donor liver (22).

Proposed solutions for the worsening shortage of organs include programs to increase society's awareness of the need to donate, the development of living donor transplantation (46), and transplantation of organs derived from animals; termed xenotransplantation (47). In spite of aggressive campaigns to educate the public, organ donation has plateaued. Application of living donation has remained limited because of the medical risks to the donor including the risk of death. To date, xenotransplantation remains experimental because of the biologic barriers of the immune system in crossing species.

Clinically, several non-biologic systems have been developed to support a failing liver in the acute setting in the hopes that the organ will regain function over time or that a suitable donor can be found given additional time (48). These systems have historically employed individual or combination components including hemodialysis; high-volume plasmapheresis/plasma exchange; hemofiltration; hemoperfusion; hemodiabsorption; and molecular adsorbent recirculating systems (48, 49). Each of these non-biologic systems will individually be discussed later given their historical significance in the developing field of bioartificial liver replacement.

Experimentally, isolated hepatocytes have been injected directly into the spleen or liver through the portal vein (50, 51). Other approaches using hepatocytes encapsulated in biocompatible matrices or attached to microcarrier beads and injected into the abdominal cavity have been reported (52-54). The success of all these cellular approaches has been limited by the relatively small numbers of cells that can be implanted. These small cell populations have not been able to demonstrate clinically significant liver specific functions. However, these studies have demonstrated that cells placed in a supportive

environment and supplied with continuous nutrients can perform cellular functions. This finding has fueled interest in the development of bioartificial liver (BAL) support devices.

Broadly defined, bioartificial liver devices contain liver cells through which the patient's plasma is perfused either with or without the associated cellular portion of whole blood. The cells modify the patient's plasma through various combinations of proteins secretion, nutritional metabolism and detoxification. To date, seven different systems have been clinically evaluated as either a "bridge to transplantation" or a temporary support for an acutely injured liver as it regains function and each approach will be discussed individually below (48, 49, 55-57).

3. NON-BIOLOGIC SYSTEMS

The only definitive treatment for ALF is orthotopic liver transplantation (46, 49, 56). However, due to the shortage of available organs for transplantation, patients with ALF may have to wait up to 10 days for an appropriate organ (49). Unfortunately, the accumulation physiologic abnormalities can rapidly lead to alteration in vascular function, acid-base balance, multi-organ system failure and ultimately death. Non-biologic liver support systems have been developed primarily to focus on the detoxification of blood in order to stabilize patients as a "bridge" to transplantation or spontaneous regeneration.

3.1. Hemodialysis

The underlying principle of hemodialysis (HD) is based on the osmotic diffusion of solutes across a semi-permeable membrane. Blood is continuously removed from the blood vessels and passed through an extracorporeal circuit including a dialyzer (Figure 1) (58). The dialyzer is composed of two chambers separated by a semi-permeable membrane. Dialysate, a sterile solution composed of various concentrations of mineral ions, flows through one chamber of the device in a counter-current direction to blood. As the blood flow through the second chamber of the dialyzer, it is cleaned by diffusion and returned to the extracorporeal circuit where it is returned to the bloodstream (59).

The mineral ion concentrations of the dialysate are designed to either promote diffusion across the membrane or prevent it. For example, the concentration of sodium and chloride are similar to those of normal plasma to prevent loss. However, urea, potassium, phosphate and other waste products, have low dialysate concentrations and readily diffuse into the dialysis solution (59). The continuous counter-current flow design maintains the concentration gradient across the membrane and maximizes the efficiency of the transfer of waste products from the blood into the dialysate. Clinically, HD is most commonly used in the setting of chronic renal failure (60).

The use of HD to treat hepatic coma was first reported by Kiley *et al.* in 1958 (7). In their study, five patients were treated with HD for ammonia intoxication

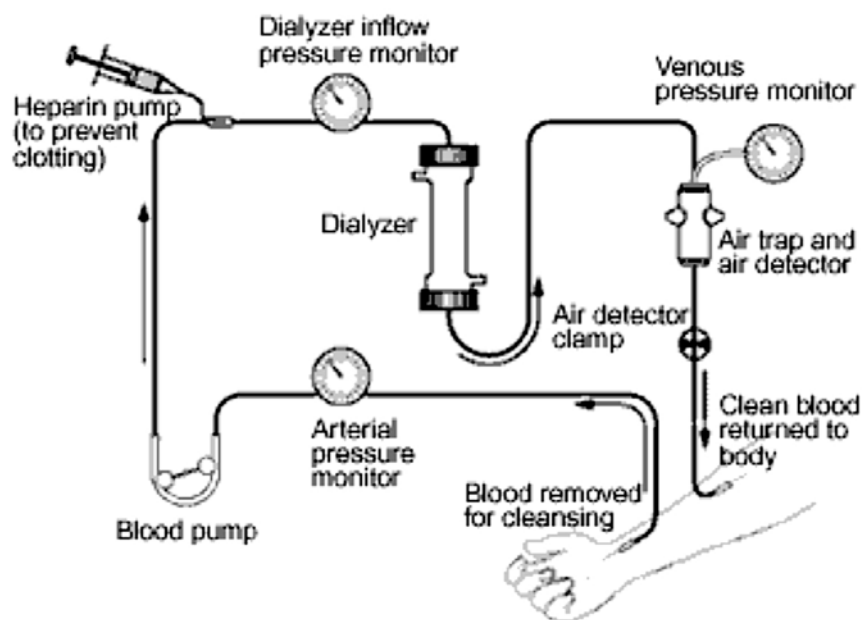


Figure 1. A Basic hemodialysis circuit. Adapted with permission from (58).

using a cellulose-membrane artificial kidney. This system allowed small molecular weight (5 kDa) toxins, like ammonia, to be removed from the blood. In each case the patient had developed ammonia intoxication following a large gastro-intestinal hemorrhage secondary to underlying chronic liver disease. In each case, HD demonstrated a decrease in the patient's arterial ammonia level. Four of the five patients showed significant neurological improvement following HD treatment, however, no long-term survival benefit was demonstrated. Each patient died within 10 days of initially becoming comatose. This failure was ultimately attributed to the artificial kidney's 5 kDa diffusion limit. It was presumed that the kidney was unable to clear so called "middle molecule" neurotoxins with weights up to 15 kDa (48).

In 1976, Knell and Dukes (61) reported a small clinical series where 4 patients with acute liver failure were treated with HD targeted towards the removal of several neuro-active amino acid precursors. The etiology of the sole patient who regained consciousness was acetaminophen-toxicity and that patient eventually made a full recovery. In that same year, Opolon *et al.* (62) reported using HD to treat 24 patients with hepatic coma secondary to viral hepatitis using a polyacrylonitrile membrane that allowed for the removal of middle molecular weight substances up to 15 kDa in size. On average, the arterial ammonia level again demonstrated a significant decrease following HD treatment. In addition, 17 of the 24 patients experienced some form of neurologic recovery, however only 5 patients in the study group eventually made a complete recovery.

While these studies showed initial promise several factors have led researchers to abandon HD as a sole therapy for treatment of ALF. First, HD is very effective at removing water-soluble toxins from the blood,

however many toxins and toxin pre-cursors in the blood are bound to serum albumin and will not diffuse across an HD membrane (63). Secondly, the only group of patients who would benefit from HD is that group whom only need acute support during an acute, but short-lived, increase in blood ammonia. The most common clinical scenario where this occurs is during a GI hemorrhage and the use of anti-coagulants for HD in such a setting is clearly undesirable (7). Finally, the long-term survival benefit conferred by HD has not been shown to be consistent or adequate in a randomized- controlled trial (49).

3.2. Hemofiltration

As in dialysis, hemofiltration involves the movement of solutes across a semi-permeable membrane. However, in hemofiltration, solute movement across the membrane is governed by convection rather than by diffusion. Hydrostatic pressure drives water and solutes across the filter membrane from the blood compartment to the filtrate compartment. All solutes are forced through the membrane at a similar rate by the flow of water in contrast to dialysis where larger solutes are removed at a reduced rate in proportion to their slower speed of diffusion (64). Unlike the episodic treatments with dialysis, hemofiltration is typically provided as a continuous treatment via double-lumen veno-venous intravenous catheters and the volume of filtrate is usually replaced with equal amounts of bicarbonate solution (34, 65).

Hemofiltration was first evaluated clinically as a treatment for ALF by Lepore *et al* as early as the 1970's (66). Over the subsequent 20 years, improvements in filtration membrane technology and equipment have significantly improved this modality's performance as a support for ALF. In 1990, Matsubara *et al* showed a 50% short-term survival in a 16-patient series where patients underwent 20 hour treatments with hemofiltration (39).

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Using high-performance liquid chromatography (HPLC) this study clearly demonstrated that hemofiltration was able to remove so-called “middle molecules” with high efficiency. Clinically, the mental status of patients improved initially as well. Unfortunately, based on prothrombin time analysis this modality did not promote liver regeneration and all but 3 of the patients in this series eventually succumbed to their liver failure. In this study, donated plasma was administered to patients as a supportive measure to address their ALF-induced coagulopathy. This practice evolved into an interest in using hemofiltration to perform high-volume plasmapheresis and replace the filtered plasma with donated plasma in large volumes (34).

3.3. High-volume plasmapheresis/plasma exchange

The liver is responsible for maintaining the proper concentrations of many serum proteins, including coagulation factors II, V, VII, IX and X (5). In the setting of ALF, production of these proteins is impaired causing a significant coagulopathy (8). Patients in ALF frequently are supported with infusions of Fresh Frozen Plasma (FFP) to address this ALF-associated coagulopathy. As outlined below, several groups have studied an extension of this supportive technique as a possible therapy for ALF through so-called “plasma exchange.” Plasma exchange involves two-steps. First the patient’s blood is separated into a cellular fraction containing red and white blood cells and a plasma fraction containing neuro-toxins and the plasma proteins including immunoglobulins and albumin. The cellular fraction is retained and mixed with donated toxin-free FFP and returned to the patient, thereby exchanging the patient’s poorly conditioned plasma for healthy plasma without significant loss of the patient’s blood cells.

Several methods have been used to separate the cellular and plasma fractions including discontinuous and continuous centrifugation, followed by plasma exchange, however these methods involved significant contamination risks and imprecise volume replacement (66, 67). Kondrup *et al.* in 1992 performed high-volume plasma exchange using a continuous filtration system capable of removing substances with molecular weight up to 500,000 (31). The system removed approximately 60% of the patient’s plasma in one pass and the volume of plasma exchanged per patient ranged from 23-77% of total body weight. 5 of 11 patients survived outright and 9 of the patients experienced significant improvements in mental status, however all of the survivors were previously healthy patients suffering from acetaminophen-toxicity. A study by Clemmesen *et al.* in 1999 demonstrated that high-volume plasma exchange improved splanchnic circulation (68). In a follow-up study in 2001, Clemmesen *et al.* demonstrated improved glutamine metabolism allowing for hepatic recovery in the setting of acetaminophen-toxicity induced ALF (32).

This modality has demonstrated its best results in acetaminophen-toxicity ALF. Despite such promising results, plasma exchange has not been demonstrated to improve long-term survival and is limited by the availability of plasma and the cost of therapy is substantial (30, 31). Further, massive plasma transfusions are fraught

with risks including hypocalcemia, metabolic acidosis, pulmonary and brain complications. While plasma exchange replaces deficient clotting factors, it also lowers blood hepatocyte growth factor (HGF) levels and is ineffective in decreasing the large intracellular pool of hepatotoxins accumulated during hepatic failure (69). In addition, this treatment requires the frequent access of an in-dwelling central catheter in patients who are already at risk for significant infections. Despite its limitations, high-volume plasmapheresis/ plasma exchange remains one of the more widely employed techniques for the management of ALF patients awaiting transplant (31, 32, 68, 70, 71).

3.4. Hemodiafiltration

When hemofiltration is used in combination with hemodialysis, it is termed hemodiafiltration. This combination is theoretically useful because it results in good removal of both large and small molecular weight solutes by taking advantage of both diffusion and convection membrane transport strategies (64). This combination strategy also showed initial promise as reported by Yoshida *et al.* in 1993 (72). Their group reported a survival rate of 55.6% in a series of 31 patients treated with hemodiafiltration for mixed etiology ALF. All patients experienced a significant improvement in neurologic function; however the patients who died failed to demonstrate liver regeneration, based on liver atrophy at autopsy examination. In a case study from 2001, Mori *et al.* reported success in decreasing direct and total bilirubin and improving prothrombin time with hemodiafiltration over 7 days, however, the patient died from respiratory complications prior to transplantation (73). In a relatively large retrospective clinical study, Sadahiro *et al.* demonstrated improved normalization of plasma using plasma exchange in concert with hemodiafiltration. Again this modality has not shown a significant long-term survival benefit, but may eventually prove useful as a short-term (i.e. 5-10 day) bridge to transplantation if studied in a randomized- controlled manner (74).

3.5. Hemoperfusion

Hemoperfusion is the circulation of a patient’s blood through a filter containing an adsorptive substance, such as charcoal, ion exchange resins or proteins (75). Of these substances, charcoal has been studied most widely and has been shown to be an effective adsorbent for many water-soluble toxins including mercaptans, gamma-aminobutyric acid, middle molecules and aromatic amino acids (76). Early studies in humans revealed that despite good clearance of toxins, direct contact between blood and charcoal adsorbents resulted in platelet activation and hemodynamic instability. One technique to address this issue is the continuous infusion of prostacyclin during hemoperfusion treatment (77). Controlled trials using this technique were conducted at the Liver Unit of King’s College Hospital by O’Grady *et al.* in the late 80’s and their results revealed “the use of charcoal hemoperfusion does not confer an additional benefit in survival over and above that obtained with intensive liver care (36).” At that time, they postulated that perhaps the adsorption of blood components was so non-selective that beneficial factors were being removed along with toxins, resulting in

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suppression of liver regeneration. As a result of these findings, hemoperfusion has been largely abandoned in favor of hemodiabsorption.

3.6. Hemodiabsorption

Hemodiabsorption is a term first introduced by Junichi Uchino at the 39th Annual Meeting of the ASAIO in 1993 (37). It refers to the process where plasma is selectively filtered through a dialyzer containing an adsorptive substance, such as charcoal, ion exchange resins or proteins in the dialysate chamber (78). The advantages of this method over hemoperfusion are three-fold: 1) the platelets are kept separated from the adsorbing compound thereby minimizing the hemodynamic effects of platelet activation; 2) the adsorbing surface area of a hemodiabsorption device could be increased by several orders of magnitude over a hemoperfusion device and; 3) hemodiabsorption devices require no more anti-coagulation than standard hemodialysis. Clinical evaluation of this system has shown promise for its use in the setting of acutely decompensated chronic liver disease, but has shown that hemodiabsorption as a stand-alone therapy confers no long-term advantage in the setting of ALF (79). In addition, several studies support O'Grady *et al.*'s assertion that the adsorption of blood components is so non-selective that factors supporting liver regeneration are removed along with toxins. When used in direct contact with the plasma, hemodiabsorption has been shown to decrease blood concentrations of hepatocyte growth factor, thyroxine, triiodothyronine, human growth hormone, and insulin (80-83). However, hemodiabsorption has found clinical application as a dialysate cleanser in the most widely tested and applied extracorporeal liver support system: the Molecular Adsorbents Recirculating System or MARS.

3.7. Molecular adsorbents recirculating system (MARS)

The Molecular Adsorbents Recirculating System (MARS) combines the use of hemodialysis to remove water-soluble toxins with the use of adsorbent substances to clear albumin-bound toxins. This novel approach is facilitated through the use of high-concentration albumin as an intermediate dialysate (38). This intermediate albumin dialysate is passed through a high-flux, albumin-impermeable hemodialyzer where diffusion of water-soluble and albumin-bound toxins are transferred to the intermediate albumin dialysate. The intermediate dialysate is first cleansed of water-soluble toxins through dialysis against a bicarbonate-based dialysate then the albumin-bound toxins are removed through hemodiabsorption in a charcoal column followed by an anion-resin exchanger. At the completion of the circuit, the cleansed albumin dialysate is recirculated through the high-flux dialyzer and the process begins anew (42).

MARS was introduced by investigators at the University of Rostock, Germany, in 1993, and extensive studies on biochemical and hemodynamic effects of this form of dialysis in 385 patients with liver failure have been reported in the literature (38, 40-42, 84). These studies have reported survival rates up to 55% in Acute-On Chronic Liver Failure (AOCLF) and ALF with no serious side

effects reported. Based on these reports MARS has been recommended for treatment of AOCLF and ALF. However, interpretation of this data is complicated by the small population studies, few prospective, randomized trials and the inability to blind these studies. Two meta-analyses have attempted to address these statistical problems. In 2004, Khuroo *et al.* performed a meta-analysis which concluded that MARS treatment conferred no significant survival benefit for patients with liver failure when compared with standard medical therapy (85). However, in the setting of AOCLF both analyses demonstrated a reduction in mortality for acute episodes of de-compensation (35, 85). Khuroo *et al.* observed that the studies to date have lacked the statistical power to demonstrate a 10% reduction in mortality, increasing the likelihood of a false-negative interpretation (85). Multi-center trials on MARS in AOCLF and ALF are ongoing and will ultimately help place MARS within the spectrum of liver support modalities.

All of these non-biologic systems share a common focus: they primarily attempt to detoxify the blood. The homeostatic metabolic and synthetic processes of the liver are merely replaced as needed via systemic intravenous infusions of blood plasma products and glucose. With no support, ALF is 100% fatal and any decrease in mortality that these devices confer beyond standard medical care should be considered an enormous success. However, in the best case scenario these devices confer a mortality rate of 50% and no improvement in long-term survival. These findings have pushed investigators to develop modalities that can replace at least some of the liver's metabolic and synthetic function while detoxifying the blood.

4. BIOARTIFICIAL LIVER SYSTEMS

The clinical experience of non-biologic systems discussed above has answered one of the essential questions concerning the development of a liver support system: metabolically active cells are an essential component for long-term liver support therapy. Demetriou *et al.* introduced the term "Bioartificial Liver" (BAL) to describe the combination of liver cells in a system with an additional artificial detoxification component in the same circuit as the liver cells (86). The first reports of a device to replace the synthetic and metabolic function of the liver were presented by Nose *et al.* in 1963, fifty years after the first successful attempts at hemodialysis for renal failure (87). The device used by Nose had a metabolic circuit containing approximately 200g of canine liver slices in warmed oxygenated media that was counter-circulated as dialysate through an artificial kidney. The artificial kidney was perfused with the patient's blood and the blood and media were kept separated by a 50 kDa gel-type cellulose membrane. This pioneering device serves to highlight many of the theoretical questions and challenges facing the development of a clinically applicable BAL. What should the source of cells be? What membrane should be used to separate the patient from the cells? Which bioreactor configuration is most clinically effective? Throughout the 4 decades since Dr. Nose's initial attempts at BAL

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replacement therapy all of these challenging issues have been studied and each issue will be reviewed here, followed by a presentation of several unique clinically-tested solutions devised by the primary groups in the field.

4.1. Cell source

Liver tissue is composed of two broad cell types: parenchymal cells and non-parenchymal cells. Parenchymal cells, or hepatocytes, are responsible for most clinically measurable organ functions including metabolic hemostasis, protein synthesis and detoxification. Non-parenchymal cells, such as stellate, Kupffer, cholangiocytes and sinusoidal endothelial cells, support parenchymal cellular orientation and function (4, 88). All clinically tested BAL's have attempted to capture the benefit of hepatocyte function by including parenchymal cells. These cells are removed from the liver through the perfusion of collagenase through the liver's vasculature releasing the hepatocytes from their extracellular matrix (89). However, hepatocytes are epidermally derived cells and require cell-to-cell contact and contact with non-parenchymal cells (90). As a result of this requirement, hepatocytes exhibit a degradation in function, loss of essential enzymatic activity and ultimately a loss of viability when isolated in culture (91). Furthermore, these systems often use conventional monolayer culture to prepare sufficient quantities of cells and these techniques poorly mimic the gradients and vectors for metabolite transfer when compared with the *in vivo* situation (4, 92).

4.1.1. Primary human hepatocytes

The preferred cells for liver replacement therapy are autologous hepatocytes. Since these cells by definition have the same immuno-markers as the patient they obviate the possibility of an immune rejection (93). Unfortunately, patients with liver dysfunction requiring liver support are in no clinical condition to withstand the removal of hepatocytes. Moreover, these diseased hepatocytes' function is needed by the patient even if barely adequate and once removed from the *in vivo* environment they may lose function entirely. Therefore the use of the patient's own cells, while immunologically advantageous, has been considered an unsatisfactory solution to the cells source problem.

The next best option for cells to populate a BAL is the use of allogenic hepatocytes; however the use of these cells is complicated by three factors. First, the only readily available source of these cells in sufficient quantity to populate a clinically effective BAL is from unused liver grafts (94). These grafts are only available sporadically and cannot be expected to be available in a timely manner for device population. Further, since the need for grafts is so great, any organ considered unfit for use in transplantation would not likely be able to withstand a preservation procedure and still retain suitable function for future application in a BAL. However, as discussed in Section 5.6, there may be new evidence to change this clinical thinking. Secondly, the use of these cells presents all the immune risks of transplantation and could complicate the patient's future rejection profile in the setting of a transplantation. Finally, since these cells are derived from

human tissue the potential for disease transmission to an immune compromised patient remains a fundamental concern (93). Despite these drawbacks, allogenic primary hepatocytes may ultimately represent the best cell population for BAL's since they initially demonstrate unimpaired cellular function (93). Attempts have been made to address the expansion and preservability of primary human hepatocyte populations through the use of immortalization techniques involving Cre-lox P mediated oncogene excision, temperature sensitive SV40Tag and suicide genes, however, none of these promising strategies have been clinically tested in a BAL (93, 95, 96).

4.1.2. Cell lines

Transformed hepatocyte cells lines which have virtually unlimited capacity for growth *in vitro* have been identified as potential sources for BAL's. These cell lines are typically derived from human hepatic tumors and demonstrate a hardy character when cultured *in vitro* (97). The most commonly used cell line in BAL research is the C3A subclone of the human hepatoblastoma (HepG2-C3A) cell line (93). This cell line was selected because it retains a wide range of normal hepatocyte functions including albumin synthesis, p450 activity, and urea metabolism despite its tumor origin (95). It also produces alpha-fetoprotein, a protein not normally produced by mature hepatocytes, which can be used as a marker of HepG2 function (97). The use of HepG2 cells is fraught with all the concerns about the use of primary human hepatocytes. In addition, since HepG2 cells are derived from a malignant tumor, a great concern exists regarding the potential transmission of metastases into immuno-compromised patients ultimately destined for transplant (94, 96). BAL's designed with these cells typically employ an extensive filtration system to prevent cells from escaping the bioreactor. BAL's containing Hep G2-C3A cells have been evaluated in early Phase I studies and have demonstrated safety with no evidence of metastases, however further testing will be required before these devices are broadly clinically applied (97).

4.1.3. Xenogenic cells

Xenogenic cells are hepatocytes derived from the livers of other animal species. Use of these cells is advantageous because they are available in virtually unlimited quantities and their production can be very tightly controlled (94). Porcine hepatocytes are especially attractive because they exhibit urea synthesis, albumin production and p450 activity very similar to human hepatocytes (93). They also tolerate a wide range of handling techniques which allow them to be procured and then stored for many months before use in a device (98). Many clinical studies have been performed using whole blood or plasma perfusion through hollow-fiber bioreactors containing freshly isolated or cryopreserved porcine hepatocytes. The devices were well tolerated by the patients but these studies did not demonstrate a survival advantage over standard of care in appropriately controlled settings (90).

One concern with the use of xenogenic cells is the transmission of zoonotic diseases, specifically porcine

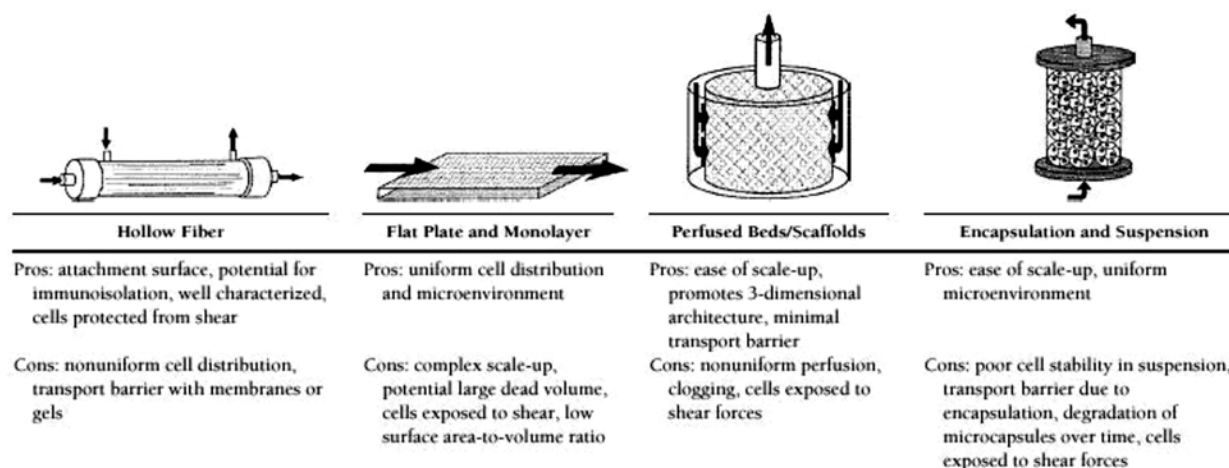


Figure 2. Bioreactor configurations. Adapted with permission from (95).

endogenous retrovirus (PERV) (90). In the mid-1990's two documents from the United Kingdom; the Nuffield Council on Bioethics' *Animal to human transplants: the ethics of xenotransplantation* and; The Advisory Group on the Ethics of Xenotransplantation's, *Animal tissue into humans* raised concerns about the transmission of zoonotic disease *in vitro* and resulted in a complete European moratorium on the clinical use of xenogenic hepatocytes (99, 100). Further European research into the applicability of xenogenic cells has essentially halted given the uncertain future of this cell source in that community. In America, xenogenic cell source research continues under the cautious guidelines of The United States Public Health Service's *Draft Public Health Service Guideline on Infectious Disease Issues in Xenotransplantation* (101). This document calls for the pre-screening of xenogenic tissues for zoonotic disease prior to use and long-term surveillance of patients in whom xenogenic cells have been implanted for signs of transmission. To date, no evidence of *in vivo* transmission has been documented. However, given the uncertainty still surrounding the use of these cells, the development of a large pool of allogenic cells would likely eliminate any further interest in this cell source option.

4.1.4. Stem cells

Stem cells are undifferentiated cells that undergo symmetric mitosis and renew their undifferentiated population while producing a daughter cell that proceeds to a committed lineage (93). Several sources of stem cells show promise for use in the field of tissue engineering at large and BAL's in particular including, embryonic, progenitor and transdifferentiated stem cells (95). However, none of these stem cell sources have been applied to a BAL and a discussion of the application of stem cells to BAL technology would be premature and beyond the scope of this discussion.

4.2. Membranes

The membrane used in the bioreactor of a BAL is expected to serve two functions. First, the membrane is expected to allow the transfer of nutrients, like glucose, and cellular products, like albumin (MW~60 kDa). However,

its second function is to prevent the transfer of albumin, immunoglobulins, complement or viruses. These molecules range in size from 150kDa to approximately 200 kDa. Therefore, most designs incorporate a membrane that has a molecular cutoff between 100 kDa and 150 kDa (95).

4.3. BAL configurations

The most common clinically applied BAL configuration involves the use of a hollow-fiber bioreactor because these designs offer enormous surface area for mass transport (96, 102). However, the performance of a hollow-fiber design as a semi-permeable membrane is poor and the bulk of the molecular filtering is done by a plasmapheresis module incorporated upstream from the bioreactor. Other configurations that have been tested *in vitro* for use in the design of a BAL include flat plate, perfused beds and encapsulation reactors. A detailed discussion of each design iteration is beyond the scope of this text however Figure 2 (95) above gives a brief synopsis of each basic configuration type and those configurations used in clinical devices will be discussed further in section 5.

5. CLINICALLY TESTED BIOARTIFICIAL LIVER ASSIST DEVICES

5.1. HepatAssist

The HepatAssist 2000 liver support system is an extracorporeal porcine hepatocyte-based bioartificial liver device manufactured by Arbios Inc. Waltham, MA. This system includes a novel open membrane hollow fiber bioreactor with approximately 7 billion cryopreserved porcine hepatocytes housed within a hollow-fiber bioreactor. The bioreactor's membrane has a pore size small enough to prevent the passage of whole hepatocytes, but large enough to allow soluble and protein-bound toxins, and large molecular weight proteins to pass through freely by fluid convection (103). These substances are exchanged between the hepatocytes in the inter-capillary space and the plasma, which travels on the inside of the fibers. The device has four components: a hollow fiber bioreactor containing primary porcine hepatocytes, two charcoal filters, a membrane oxygenator, and a pump (98).

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Initially the patient's blood is separated into plasma and cellular components in a plasmapheresis device. The plasma is passed through two charcoal filters, which detoxify the blood in a fashion similar to a hemodialysis system. After initial detoxification, the plasma runs through the hepatocyte-lined hollow fiber column, where the hepatocytes further condition the plasma through the removal of ammonia, lactate and bile acids and the secretion of albumin, and glucose (103, 104). The newly conditioned plasma is then combined with the cellular fraction and the reconstituted whole blood is returned to the patient. During the process, a membrane oxygenator and heater, in series between the charcoal filters and hepatocyte bioreactor, keep the plasma and the hepatocytes oxygenated at body temperature (98).

This system was initially developed by a group at Cedars-Sinai Medical Center in Los Angeles, CA and is the most extensively studied of the extracorporeal cell-based liver support systems (103). The device was initially evaluated in a series of *in vitro* and *in vivo* pilot clinical studies with consistent improvements in neurologic function warranting more rigorous clinical evaluation (105). In 1997, the device was evaluated in phase I/II clinical trials which demonstrated safety and showed encouraging signs as either as a bridge to transplantation or recovery of normal liver function (103). The device was then evaluated in a 4-year randomized-controlled trial which completed enrollment in 2001. The trials treated 171 patients at 20 clinical centers in the US and Europe and demonstrated an improved survival benefit for patients with fulminant and sub-fulminant liver failure (90). However, the device's future has been uncertain since the FDA determined that the study failed to clearly demonstrate the device's overall efficacy. This ruling essentially demands a full phase III efficacy trial in order for the device to be approved for clinical application, a difficult task to accomplish given the study size required to demonstrate a certain benefit (98).

5.2. Extracorporeal liver assist device (ELAD)

Vital Therapies' ELAD® device is similar in concept to the HepatAssist system, however the ELAD® uses HepG2-C3A hepatocytes instead of porcine liver cells (106). The system was designed by a group at Baylor Medical School in Houston, TX (107) and is composed of 4 hollow fiber dialyzer cartridges (Figure 3).

Prior to use, each cartridge is seeded with a small quantity of cells and the cell population is allowed to "mature", or grow to confluency (108). During a three-week maturation process, the cells replicate and attach to the outside of the cartridge's capillaries. At maturity each cartridge contains approximately 200g of hepatocytes (97, 106, 108). A continuous veno-venous hemofiltration machine forms ultra filtrate at 400-900 ml/hr which is pumped through an oxygenator and then is perfused through 4 mature cartridges (109). This filtration scheme confers a direct benefit over the HepatAssist device in that it can be run continuously for up to 10 days without interruption (107). The ultrafiltrate is passed through a 1 micron filter before being reconstituted to whole blood to

ensure that no HepG2 cells enter the patient's bloodstream (108).

Twenty-four patients in two clinical trials were treated with the initial prototype with promising results as measured by several clinical indicators including; Mean Arterial Pressure, Cardiac Index and Systemic Vascular Resistance. Neurologic function was maintained or deteriorated more slowly in the study group when compared to controls (109). The device was then evaluated in Phase I and Phase II trials enrolling a total of 44 patients at twelve centers in the United States and England. These trials demonstrated the safety of the ELAD® System and the clinical data showed improvement over controls in bridge to transplant, survival and withdrawal of treatment as futile (107). Enosawa *et al.* have recently increased the HepG2-C3A cell line's ammonia clearance capacity through a transfection with glutamine synthetase (110). The ELAD® is currently being evaluated in a pivotal clinical trial in China for acute-on-chronic liver failure and these results will direct future studies under the auspices of the U.S. FDA (107).

5.3. Bioartificial liver support system (BLSS)

The Excorp Medical Bioartificial Liver Support System (BLSS) uses a culture of primary porcine hepatocytes housed in the extra-luminal space of a hollow-fiber bioreactor. The hepatocytes are infused into the bioreactor following harvest and are maintained under tissue culture conditions until needed (111, 112). It was designed by a group at the University of Pittsburgh in association with the McGowan Institute for Regenerative Medicine. Both the BLSS and the HepatAssist system are porcine based systems and the primary difference between them is that the BLSS allows whole blood to directly perfuse the luminal space of the hollow fiber cartridge (112). Similar to the ELAD system, the BLSS does not use a charcoal absorbent system to detoxify the blood prior to perfusion into the bioreactor (113). All three systems shared an oxygenator as a common component directly preceding the bioreactor in each circuit (Figure 4).

Each BLSS treatment lasts for approximately 36 hours consisting of a 12-hour baseline monitoring period, a 12-hour perfusion period with the bioartificial liver support system (BLSS), and a 12-hour post perfusion baseline monitoring period (111). Blood passes through a heat exchanger and oxygenator and then through the hollow-fibers of the bioreactor containing a 70–100 g culture of primary porcine hepatocytes (113). The fibers prevent the patient's blood from directly contacting the cells but allow for diffusion of toxins (111). After passing through the BLSS one-time the clean blood is pumped back into the patient.

The first clinical use of the BLSS was to support a 41-year-old female with fulminant hepatic failure (114). BLSS treatment in this patient was associated with improvements in ammonia and lactate concentrations, reductions in total bilirubin reduction, and improvement in coagulopathy as measured by prothrombin time. In addition the patient's overall clinical picture improved to the extent



Figure 3. An ELAD® cartridge. Adapted with permission from (107).

that she was successfully extubated. A study published in 2001 by Mazaregios *et al.* assessed the safety of treating 4 patients that had acute liver failure (115). All four tolerated treatment well, showing mild neurological improvement and moderate reduction in blood ammonia levels. While safety was clearly demonstrated, this device demonstrated the same deleterious effects seen as a result of platelet activation in hemoperfusion systems, including hypotension, thrombocytopenia, coagulopathy and hypoglycemia (112, 114).

As with all other porcine based systems this device has been evaluated for transmission of PERV. In 2002, Kuddus *et al.* evaluated the safety of BLSS treatment with respect to transmission of Porcine Endogenous Retrovirus (PERV). Based on serial examinations of blood and bioreactor effluent from 5 patients treated with BLSS they demonstrated that a semi-permeable membrane with a 100-kDa nominal cutoff is adequate to prevent transmission of PERV at 3, 6, and 12 months post-perfusion (116).

5.4. TECA-hybrid artificial liver support system (TECA-HALSS)

Several groups in China have been studying a device referred to in the literature as the TECA Hybrid Artificial Liver Support system (TECA-HALSS). This system bears many similarities to the Hepatassist 2000 including a hollow-fiber bioreactor, an oxygenator/heater, and a charcoal absorption column. However, the primary distinction is the use of a TECA Bioartificial Liver Support System (TECA-BLSS) module (TECA Corp. Hong Kong, PRC) containing a hepatocyte cell suspension including growth factors. This cell suspension and nutrient slurry is perfused through the outer space of the hollow-fiber bioreactor against an intra-luminal counter flow of plasma previously treated by charcoal absorption (117, 118). A typical TECA-HALSS treatment lasts 6 hours followed by a plasma exchange with volumes ranging from of 2-3 liters (117), in one study to as high as 3.5-4 liters (102).

In 2001, Xue *et al.* treated 6 patients with acute and chronic liver failure using the TECA-HALSS and reported very promising results (117). No tachypnic, hyoptensive or tacycardic events were reported, and all subjects experienced an improvement in their neurologic function. In addition, total and direct bilirubin and ammonia all trended towards normal, while an improvement in PT was noted in all subjects. Xu *et al.* report two typical cases where patients were treated for hepatic encephalopathy with TECA-HALSS and were so significantly improved that they left the hospital within 8

days (117). In a report published by Ding *et al.* in 2003, 9 of 12 patients showed significant improvement in hepatic function and, hepatic encephalopathy when treated with the TECA-HALSS system (119). As with other devices, the TECA-HALSS has yet to be tested through a rigorous prospective randomized clinical trial and its future use will be dictated by further application with much larger study sizes.

5.5. AMC- bioartificial liver (AMC-BAL)

The Academic Medical Center Bioartificial Liver (AMC-BAL) is a device that combines a hollow-fiber configuration with a perfused bed configuration (120) and was developed by a group at the University of Amsterdam, The Netherlands (121). The bioreactor is housed in a polysulfon dialysis chamber that contains multiple polypropylene oxygenation tubes running longitudinally. Warmed oxygen is passed through these tubes from the oxygen inlet to the oxygen outlet. The oxygenation tubing is wrapped by a non-woven polyester fabric onto which porcine hepatocytes are seeded. A plasmapheresis system is used to separate the patient's plasma from the cellular fraction of the blood and the plasma is pumped in direct contact with the cell within the bioreactor from the plasma inlet side-port to the plasma outlet side-port. This configuration was adopted to allow high-density hepatocyte cultures to form more physiologically-sized aggregates (121) after it was observed that hepatocytes in hollow-fiber bioreactors formed large aggregates that limited mass transfer to the cells at the center of the aggregate. After recirculation through the bioreactor, the plasma is reunited with the blood cells from the plasma-separator and returned to the animal (122).

This device was tested in several animal studies in the late 1990's and completed a phase I trial which was reported by van de Kerkhove *et al* in 2002 (123). This study enrolled a total of seven patients with ALF of mixed etiology and all patients showed prompt improvement of neurologic function following treatment with associated improvements in bilirubin and ammonia concentrations. Two of the seven patients in this study died as a result of complications of transplantation however, all patients were tested for the presence of PERV and anti-porcine antibodies with no findings. A case report by van de Kerkhove *et al* in 2004 demonstrated the successful bridging of a patient to transplant using an AMC-BAL (49). Several modifications and examinations of this system have been reported in the literature including comparisons of seeding density and cell type; different materials; and an analysis of oxygen availability using computational fluid dynamics (120, 124). In 2001, Calise *et al.* presented a plan for a multi-center clinical trial in Europe however European Legislation currently prohibits the use of porcine hepatocytes in BAL devices (125). The system is currently being adapted for use with human hepatocytes (126).

5.6. Modular extracorporeal liver system (MELS)

A group at the Charite Virchow Clinic in Berlin has designed the Modular Extracorporeal Liver System (MELS) (127) which as the name implies is composed of modules that can be tailored to individual patient's needs.

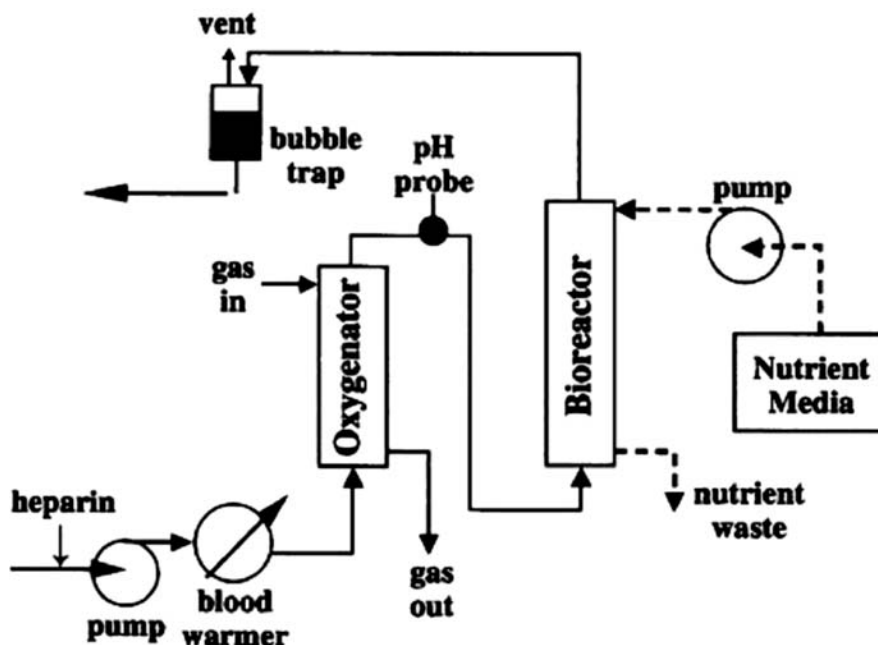


Figure 4. Schematic flow diagram of Excorp Medical, Inc bioartificial liver support system (BLSS). Adapted with permission from (114).

Modules include a blood module; a detoxification module; a liver cell module; and a dialysis module (Figure 5) (128).

The CellModule (Figure 6) is composed of three independent, interwoven capillary systems which provide medium inflow, cell oxygenation/carbon dioxide removal, and medium outflow. Two sets of Polyether-sulphone (PES) hollow-fiber membranes are incorporated into the bioreactor. By closing one end of each set, plasma entering the reactor via one set must enter the extra-capillary space before leaving the reactor, via the second set of membranes (92). The third set of hollow fibers is made of hydrophobic membranes that are used for rapid oxygenation and carbon dioxide removal. Cells are seeded in the extra-capillary space and the interwoven design of these three hollow-fiber systems forms a three-dimensional capillary network with high performance mass exchange for nutrient and substrate supply (128).

Initially this system was tested using primary porcine hepatocytes co-cultured with non-parenchymal cells as reported by Mundt *et al.* in 2002 (129) and Sauer *et al.* (130). In the study by Mundt, the CellModule was seeded with between 180 and 550 g of freshly isolated porcine hepatocytes which were incubated with medium until needed for clinical application. All seven patients were successfully bridged to transplantation and at two-year follow-up the study reported 100% survival. This study took the novel approach of calculating the ammonia uptake and urea release for each bioreactor since the concentration of these substances in the patient's blood can be effected by renal and other organ failures. They showed a net positive ammonia uptake in 5 of 7 bioreactors and a continuous release of urea in 6 of 7 reactors, indicating a trend towards normal liver function (129). The performance

of this system in phase I trials indicated its safety as no patients had an adverse reaction to treatment. After 3 to 4 years of follow-up, no PERV infections have been detected (130). Despite this finding, this group has elected to pursue the use of discarded human liver from transplant programs as an alternative to the use of xenogenic liver cells to charge the bioreactor (130).

In a study from Gerlach *et al* in 2003, primary human hepatocytes and non-parenchymal cells were isolated from discarded human liver grafts and used to seed the MELS CellModule (131). The CellModules were incubated in culture over several weeks and the metabolic activity of the cultures, as measured by urea synthesis, ammonia detoxification, galactose, and sorbitol uptake, was used to indicate stable performance. Microscopy revealed that cell aggregates partially formed parenchyma-like structures in bioreactors containing between 30% and 80% of well-preserved hepatocytes. This study clearly indicated that primary human hepatocytes from discarded transplants do recover from injury and can be maintained in bioreactors, suggesting that these cells may develop into a clinically viable cell source for BAL's. Despite these promising findings, this device has not completed a phase III clinical trial and will require further clinical testing for efficacy.

5.7. Radial flow bioreactor (RFB)

A group from the Jikei University School of Medicine in Tokyo, Japan and a group at the University of Ferrara in Ferrara, Italy have independently investigated a radial flow bioreactor (RFB) (132, 133). These two devices are similar to one another however the Japanese device employs human hepatocellular carcinoma cells whereas the Italian device employs freshly isolated porcine hepatocytes.

The promise of bioartificial liver replacement

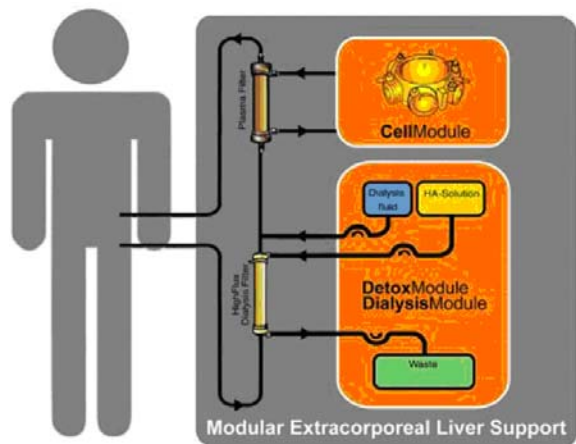


Figure 5. A Schematic diagram of the MELS system. Adapted with permission from (128).



Figure 6. A cut-away view of the MELS CellModule. Adapted with permission from (128).

The Italian device has proceeded further in testing and is briefly presented here. The bioreactor is a perfused bed configuration using a 6-mm thick woven-non-woven polyester fabric wrapped in a coaxial cylindrical shape within a polycarbonate column. The fabric is seeded with 200-230 grams of freshly isolated hepatocytes, and warmed, oxygenated plasma is flowed through the fabric from the periphery to a central collecting chamber where it exits the bioreactor and is reconstituted to whole blood and returned to the patient (133). In 2002, Morisani *et al.* reported a phase I study where 7 patients were treated with the Italian RFB for ALF of mixed etiologies (133). Six of these patients were successfully stabilized and bridged to transplantation. One patient succumbed to multi-organ system failure. Patients were screened for PERV transmission at the time of treatment, 30 and 180 days with no evidence of transmission found. Despite the fact that this system performed well in phase I trials, the future of this European system remains uncertain based upon the need for further clinical tests and the current prohibition on the use of porcine hepatocytes in European Clinical trials.

6. FUTURE DIRECTIONS

The future of bioartificial liver replacement remains bright, however many advancements will be

required before this technology will be readily applicable in the clinical setting. The optimum cell source has yet to be identified and this central issue will require improvements in long-term *in vitro* cellular stability and viability. Current bioreactor designs have only recently begun to address the interactions between hepatocytes, non-parenchymal cells and the extracellular matrix where optimization of this process may lead to vast improvements in performance. Finally, future bioreactors will need to recapitulate the three-dimensional architecture of the liver through the use of developing manufacturing techniques to maximize the mass transfer of nutrients and oxygen.

The optimum cell source for BAL's has yet to be identified and issues surrounding augmentation, expansion, storage and co-culturing remain unresolved. Several groups have investigated the use of augmented cells through genetic modifications to enhance the performance of hepatocytes within a bioreactor such as the addition of glutamine synthetase to HepG2 cells to improve the metabolism of ammonia (110), or the transfection of an IL-1 receptor agonist to porcine hepatocytes to attenuate the effect of IL-1 in the setting of ALF (134). Several genetic modifications have also been suggested to allow small cell populations to be expanded to clinically sufficient quantities, including the use of SV40 and Cre/loxP-mediated excision to induce reversible immortality in primary human hepatocytes (96). In addition, the handling and storage conditions for these sensitive devices present significant logistical problems which will require the development of new techniques like the strategy of shipping pre-seeded BAL's under hypothermic conditions similar to today's liver transplant grafts (135). Several *in vitro* studies have indicated that the importance of non-parenchymal cells may have previously been dramatically underestimated and defining the correct co-culturing strategy for BAL's will be an area of growing interest (131, 136). Genetic modifications may allow for augmentation and expansion of hepatocyte populations and co-culturing of hepatocytes with non-parenchymal cells can improve cell-to-cell interactions, however several issues of bioreactor design need to be addressed to support these cells long-term.

Today's bioreactor designs have become quite sophisticated however they fail to recapitulate many aspects of normal liver architecture that may prove vital to future success. All the clinically-tested devices described above require that hepatocytes attach to synthetic surfaces and their ultimate function may be improved through the use of more bio-mimetic substrates such as collagen (137). Along the length of a liver sinusoid, hepatocytes in the liver are faced with gradients of oxygen and nutrients that vary according to their distance from the portal inlet and the venous outlet. As a result of these gradients, hepatocytes display varying function along the length of the sinusoid (4). Future bioreactors may need to recreate this microenvironment in order for their hepatocytes to eventually assume all the functions of a normal liver (95, 96, 138). One way to achieve this effect may be through the use of a developing manufacturing technology called three-dimensional printing. This nascent technology has only

recently been investigated for applicability in bio-systems however several groups have been successful in applying this technology to the precise placement of cells with a matrix (139, 140). Ultimately, the incorporation of a pre-formed vascular network may be required for bioartificial liver replacement therapy to reach its final goal: an implantable device made of completely resorbable materials for permanent liver replacement (141).

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