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Bio-artificial liver: An advanced therapy for liver failure

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Abstract
Liver failure is one of the major cause of mortality worldwide. Without liver transplantation there was no other way for survival of patient suffering from liver disease either acute or chronic. Two types of devices are now available for temporary support- artificial and bioartificial liver. Artificial liver generally use non-living components to remove toxins accumulated during liver failure. Bio artificial livers use bioreactors containing porcine or human hepatocytes to provide biotransformation and synthetic liver functions. Different bio artificial liver systems are being investigated clinically on the basis of their capacity to provide and replace most of the liver functions. However, there are some challenges such as cost, cell availability and maintenance of cell viability to be overcome. But despite all of these, bioreactors, when combined with artificial components, becomes a pragmatic approach for future treatment of liver failure.

Keywords: Bio-artificial Liver, Importance, Component, Bioreactor.

Introduction
A bio artificial liver device (BAL) is an artificial extracorporeal supportive device for an individual who is suffering from acute liver failure. The liver is a complex organ with various vital functions in synthesis, detoxification and regulation; its failure therefore constitutes a life threatening condition. Liver failure (LF) can either occur without preceding liver disease (acute liver failure, ALF), usually caused either by intoxication or as acute decompensation of chronic liver-related illness (acute-on-chronic liver failure, AoCLF). In both cases, its symptoms include hepatic encephalopathy and impairment of coagulation status and may result in multi organ failure (Gimson *et al*., 1982) [6].

The only long-term therapy in most cases is orthotopic liver transplantation, unless the liver is able to regenerate. Many patients, especially those who are not listed for high urgency transplantation, may not survive until a suitable donor organ is available, since donor organs are rare. In other cases, contraindications do not permit liver transplantation. For these indications, extracorporeal liver assist devices have been developed in order to either bridge the patient to transplantation or temporarily support the failing organ until it is able to regenerate (Doria *et al*., 2006) [7].

Development
The first bioartificial liver device was developed by Dr. Kenneth Matsumara and was named an invention of the year by Time magazine in 2001. Liver cells obtained from an animal were used instead of developing a piece of equipment for each function of the liver. The structure and function of the first device resembles that of today's BALs. Animal liver cells are suspended in a solution and a patients blood are separated by a semipermeable membrane that allow toxins and blood proteins to pass but restricts an immunological response. Advancements in bioengineering techniques have led to improved membrane constructs and hepatocyte attachment systems. As time has progressed the sources of hepatocytes have increased. Cell sources now include primary porcine hepatocytes, primary human hepatocytes, human hepatoblastoma (C3A), immortalized human cell lines and stem cells (Allen *et al*., 2001) [5].

Cells used in bio artificial liver system

The full complement of cellular functions required in BAL devices to effect positive clinical outcomes has not been determined. To address this problem, surrogate markers of each class of liver-specific functions typically are characterized including: synthetic, metabolic, detoxification (phase I and II pathways), and biliary excretion. Primary porcine hepatocytes are most commonly used in devices undergoing preclinical and clinical evaluation. Studies have also been conducted with cells isolated from rabbit, canine, and rodent species. There is relatively limited information on the maintenance of liver-specific functions of porcine hepatocytes *in vitro*. Although some functions such as albumin secretion may be stable, others such as cytochrome P450 decline under standard culture conditions. In general, primary hepatocytes are well known to require specific micro environmental cues to maintain the hepatic phenotype *in vitro*, and it is likely that a more detailed investigation of culture conditions will improve the stability of porcine hepatocytes *in vitro* as has been the case for rodent hepatocytes (Roger *et al.,* 1998) [11]. Primary human cells would be ideal, but like whole organs, they are in limited supply. They have been used for BAL application as well as for hepatocyte transplantation. A persistent paradox of human hepatocytes is their facile proliferation in vivo but static nature in culture, despite significant progress in stimulating DNA synthesis of rodent hepatocytes in culture. Recent reports regarding underlying differences in telomerase expression in humans and rodents may play a role in this phenomenon. The growth limitations of primary cells has spurred attempts to develop cell lines that can proliferate in culture while maintaining liver-specific functions. Many cell lines have been established by retroviral transduction or lipofection of the simian virus 40 tumor antigen gene (SV40Tag) whose gene product binds to cell cycle regulator proteins Rb and p53. Spontaneous immortalization has been documented as a result of collagen gel sandwich cultures or cocultures. Cell lines derived from hepatic tumors, such as C3A (a subclone of HepG2), have already been used in clinical trials (Behnia *et al*., 2000) [8].

Important features of bio artificial liver system

1) Cellular components must be purified and every component in it must be clearly identified.
2) The cellular preparation must be clearly shown to not transmit any infectious diseases of any kind.
3) The cellular component must stay viable and active.
4) The synthetic component must be fully biocompatible, integrity of the material and parts must also be demonstrated.
5) The device must be able to introduce the therapeutic and regulatory molecules that a healthy liver provides, and it must also filter substances from the blood the way that the normal liver does.
6) Must be immunocompatible.
7) Blood must perfuse properly through this system (Allen *et al*., 2001) [5].

Bioreactor

A bioreactor may refer to any manufactured or engineered device or system that supports a biologically active environment. In one case, a bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from litres to cubic meters, and are often made of stainless steel (Doran, 2013) [9].

A bioreactor may also refer to a device or system meant to grow cells or tissues in the context of cell culture. These devices are being developed for use in tissue engineering or biochemical engineering.

Bioreactor design

In normal liver, no hepatocyte is farther than a few micrometers from circulating blood; thus, transport by diffusion has to occur only over very short distances. Although oxygen diffusivity is an order of magnitude greater that that of many other small metabolites (e.g., glucose and amino acids), it has very low solubility in physiological fluids deprived of oxygen carriers. Thus, itis not possible to create large concentration gradients, which would provide the driving force for rapid oxygen transport over long distances. This, in addition to the fact that hepatocytes have a relatively high oxygen uptake rate, makes oxygen transport the most constraining parameter in the design of BAL devices.

A successful and clinically effective BAL device should satisfy a few key criteria: adequate bidirectional mass transport, maintained cell viability and function, and potential for scale-up to therapeutic level-

1. Bidirectional mass transfer is one of the most important criteria in BAL to provide nutrients to sustain cell viability and allow export of therapeutic cell products. Semipermeable membranes provide selectivity for the size of biological molecules that will be exchanged between the patient and the device.
2. Cell viability is another criteria for bio artificial liver reactor generation. Bioreactor provide microenvironment for hepatocytes, but do very little for cell viability.
3. For a device to become a clinical reality, it must be scaled to a size that provides effective therapy. Studies indicate that between 10% and 30% of normal liver mass is needed to sustain life, which in adults, corresponds to 150 to 450 g of cells. Clinically tested devices incorporate between 1 and 500 g of hepatocyte mass (Allen *et al*., 2001) [5].

Bio artificial liver systems

BALs are essentially bioreactors, with embedded hepatocytes that perform the functions of a normal liver. They process oxygenated blood plasma, which is separated from the other blood constituents. Several types of BALs are being developed, including hollow fiber systems and flat membrane sheet systems.

Hollow fibre system

One type of BAL is similar to kidney dialysis systems that employ a hollow fiber cartridge. Hepatocytes are suspended in a gel solution, such as collagen, which is injected into a series of hollow fibers. In the case of collagen, the suspension is then gelled within the fibers, usually by a temperature change. The hepatocytes then contract the gel by their attachment to the collagen matrix, reducing the volume of the suspension and creating a flow space within the fibers. Nutrient media is circulated through the fibers to sustain the cells. During use, plasma is removed from the

patients blood. The patient's plasma is fed into the space surrounding the fibers. The fibers, which are composed of a semi-permeable membrane, facilitate transfer of toxins, nutrients and other chemicals between the blood and the suspended cells. The membrane also keeps immune bodies, such as immunoglobulins, from passing to the cells to prevent an immune system rejection.

The hollow-fiber system has been the most widely used type of bioreactor in BAL development. The hollow fiber cartridge consists of a shell traversed by a large number of small-diameter tubes. The cells may be placed within the fibers in the intracapillary space or on the shell side in the extracapillary space. The compartment that does not contain the cells is generally perfused with culture medium or with the patient's plasma or blood. The fiber walls may provide the attaching surface for the cells or act as a barrier against the immune system of the host. Microcarriers have also been used as a means to establish an attachment surface for anchorage-dependent cells introduced in the shell side of hollow-fiber devices (Bhatia *et al*., 1999) [10].

Molecular adsorbents recirculating system (MARS)

Molecular adsorbents re-circulating system (MARS), a haemodialysis and haemofiltration device, for the treatment of liver failure. The MARShas been available in Australia since 2002. The molecular adsorbents recirculating system (MARS) is a blood detoxification system based on albumin dialysis indicated for patients with ALF (acute liver failure) and CLF (chronic liver failure). The system removes both protein-bound and water-soluble toxins, which makes it useful for patients with liver failure complicated by renal insufficiency. The aim of MARStherapy is to provide support of the liver until recovery or as a bridge to transplantation. The principle mechanism of action in MARStherapy is hemadsorption, which combines haemodialysis with adsorption using albumin (Sen & Jalan, 2004) [1].

The MARSsystem consists of three compartments – a blood circuit, an albumin circuit and a renal circuit. Blood flows through the dialysis module, crossing an albumin-impregnated dialysis membrane. The albumin circuit is filled with 20% human albumin solution, which acts a dialysate. The albumin is pumped through the MARS membrane compartment counter current to the blood flow. Protein-bound toxins and water-soluble substances diffuse into the albumin solution. The albumin dialysate is then passed through an additional dialysis membrane, counter-current to a standard dialysis solution where diffusive clearance or water-soluble substances occurs. The solution is then cleared of its albumin-bound toxins by passage through an activated carbon adsorber and an anion exchanger albumin-bound toxins by passage through an activated carbon adsorber and an anion exchanger, (Sen & Jalan 2004) [1]. Treatment duration varies between 6-24 hours (Sen & Mookerjee, 2002) [2].

Extracorporeal Liver Assist Device (ELAD)

ELAD device is hepatassist device. ELAD uses a line of cultured human hepatocytes instead of porcine liver cells. The device is a "metabolically active" hollow fiber dialyzer analogous to cartridges used in kidney dialysis. The dialyzer is a two-chambered canister, mechanically very similar to a kidney hemodialyzer – like a container full of microscopic straws. The dialyzer cartridge's extra capillary space is inoculated with a patented, cloned, immortalized 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 cartridge's capillaries. The dialysis unit removes blood from the patient, pumps it into the ultrafiltrate generator, where it is separated into a cellular component and a plasma component. The plasma is circulated through the ELAD cartridge, or cartridges, then recombined with the cells and returned to the patient. The ELAD cartridge is a disposable single-use device.

Bioartificial Liver Support System (BLSS)

The BLSS is an extracorporeal hemofiltration device. It contains a hollow fiber membrane (with 100kDa cutoff) bioreactor that separates the patient's blood from approximately 100 grams of primary porcine hepatocytes that have been harvested from purpose-raised, pathogen-free pigs. The actual BLSS device consists of a blood pump, heat exchanger to control blood temperature, an oxygenator to control oxygenation and pH, a hollow fiber bioreactor and associated pressure and flow alarm systems. The treatment lasts for approximately 12 hours. Blood is pumped from a patient's venous system via a catheter into a bioreactor — or canister — outside the body. Blood passes though a cylinder filled with hollow polymer fibers and a suspension containing billions of pig liver cells. The fibers act as a barrier to prevent proteins and cell byproducts of the pig cells from directly contacting the patient's blood but allow the necessary contact between the cells so that the toxins in the blood can be removed. A heat exchanger and oxygenator are also housed in the unit to control blood temperature, oxygenation, and pH. Pressure and flow alarm systems indicate any significant fluctuation in pressure or flow. After having passed through the BLSS, clean blood is pumped back into the patient (Mazariegos *et al.,* 2001) [3].

The modular extracorporeal liver support (MELS)

The modular extracorporeal liver support (MELS) system was designed by the Charité Virchow Clinic in Berlin. This device consists of 3 interwoven capillary bundles for hepatocyte culture in a polyurethane housing. These capillary bundles serve to oxygenate and perfuse the cell culture with patient plasma. A unique feature of the MELS system is that it uses primary porcine hepatocytes as well as human hepatocytes isolated from livers unsuitable for transplantation. This module is combined with SPAD and continuous veno-venous hemodiafiltration techniques for improved efficacy. The combination of approaches puts fewer burdens on the hepatocytes. A phase 1 clinical study on 8 patients was performed using porcine hepatocytes and 12 patients are currently being treated with primary human hepatocytes in another clinical trial.

Conclusion

The extracorporeal bioartificial liver is a promising technology for the treatment of liver failure, but significant technical challenges remain before systems with sufficient processing capacity and of manageable size can be developed. Most efforts to date have focused on device design and construction, and more recently on the development of methods to generate a continuous supply of human hepatocyte cell lines. New designs are not yet able to

stably sustain the large cell populations needed for therapeutic purposes, and concerns about the safety of transformed cell lines remain. On the other hand, there have been fewer efforts to improve the specific functional capacity of the hepatocytes used in bioartificial livers. Increasing the efficiency of the cellular component of bioartificial livers would greatly facilitate their design. The MARSdevice has been granted 510K pre-market approval from the FDA in June 2005. The functional capacity of cells used in bioartificial livers may be improved on at least three different levels: 1) by altering the internal machinery of individual cells to upregulate critical functions; 2) by judiciously controlling the spatial distribution of multiple cell populations with different specializations to emulate the organization of the liver acinus; and 3) by optimizing the overall treatment protocol, including the interval, duration, and number of treatments.

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