**REVIEW ARTICLE**

**Bioartificial Liver - Bridge To Liver Transplantation**

Pallavi M. Nigade*, Swapnil L. Patil and Pournima R. Shinde

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**Abstract:** The liver is a vital organ present in vertebrates and some other animals; it has a wide range of functions, a few of which are detoxification, protein synthesis, and production of biochemical necessary for digestion. The liver is necessary for survival; a human can only last up to 24 hours without liver function.

The purpose of study is to develop bioartificial liver device to treat patients with severe liver disease until they can be transplanted or recover spontaneously. Bioartificial liver device is a supportive device, either allowing the liver to regenerate properly upon acute liver failure, or to bridge the individual’s liver functions until a transplant is possible. The technical and clinical objective is to provide a temporary liver assist. The ultimate objective of this work is the study of a system which removes toxins effectively.

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**INTRODUCTION**

**Liver**

The liver plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, and detoxification. The liver is also the largest gland in the human body. It lies below the diaphragm in the thoracic region of the abdomen. It produces bile, an alkaline compound which aids in digestion, via the emulsification of lipids. It also performs and regulates a wide variety of high-volume biochemical reactions requiring very specialized tissues.

**Significance of Bioartificial Liver**

Bioartificial livers are intended to be a “bridge” to liver recovery or transplant. Such systems can also help stabilize a patient condition after a transplant. These systems serve as a temporary liver support while allowing the liver to regenerate on its own or until a suitable organ becomes available for transplantation when liver can no longer perform its functions. BALs are essentially bioreactors, with embedded hepatocytes (liver cells) that perform the functions of a normal liver. They process oxygenated blood plasma, which is separated from other blood constituents. Several types of BALs are being developed, including hollow fiber systems and flat membrane sheet systems.

**LIVER TRANSPLANTATION**

**Definition:** Liver transplantation is a surgery that removes a diseased liver and replaces it with a healthy donor liver.

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There are four types of liver transplantation methods.

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* Pad. Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune, Maharashtra, India 411018, E-mail: swapnilpatil.mun@gmail.com

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(1) Orthotropic Transplantation
It is the replacement of a whole diseased liver with a healthy donor liver. When an orthotropic transplantation is performed, a segment of the inferior vena cava attached to the liver is taken from the donor as well. The same parts are removed from the recipient and replaced by connecting the inferior vena cava, the hepatic artery, the portal vein and the bile ducts.

(2) Heterotopic Transplantation
It is the addition of a donor liver at another site, while the diseased liver is left intact. When there is a possibility that the afflicted liver may recover, a heterotopic transplantation is performed. The donor liver is placed in a different site, but it still has to have the same connections. It is usually attached very near the original liver, and if the original liver recovers, the donor shrivels away. If the original liver does not recover, it will shrivel, leaving the donor in place.

(3) Reduced-size Liver Transplantation
It is the replacement of a whole diseased liver with a portion of a healthy donor liver. Reduced-size liver transplants are most often performed on children. Reduced-size liver transplantation transplants part of a donor liver into a patient. It is possible to divide the liver into eight pieces, each supplied by a different set of blood vessels. Two of these pieces have been enough to save a patient in liver failure, especially if the patient is a child. It is therefore possible to transplant one liver into at least two patients and to transplant part of a liver from a living donor and have both donor and recipient survive. Liver tissue grows to accommodate its job so long as there is initially enough of the organ to use. Patients have survived with only 15-20% of their original liver, provided that 15-20% was healthy.

(4) Bioartificial Liver
It is used for a short period of time when there is no presence of donor liver. Artificial livers are intended to be a “bridge” to liver recovery or transplant. Such systems can also help stabilize a patient condition after a transplant. It is used to remove toxins from the blood, capacity to maintain multiple liver functions such as protein synthesis, enzyme activity and drug metabolism, replace lost liver functions.

BIOARTIFICIAL SYSTEMS
The system such as filtration/dialysis can remove toxic substances from the blood but, they cannot replicate other functions of liver, including protein synthesis. To improve this or to support systems there is utilization of actual animal or human liver cells. So in artificial systems are hybrids that also have filtration/dialysis capability.

The core component of a bioartificial liver system is “bioreactor”, which contains active liver cells. Most systems use hollow fiber capillaries, but some newer systems use various other configuration. A membrane typically separates the patients plasma from the liver cells, but allows an exchange of toxins and other substances. As they do in the body the liver cell take in oxygen and nutrients, and return metabolic byproducts to plasma.

The first bioartifical system used rabbit cells, but today’s system use pig hepatocytes as cloned human liver cells. It is estimated that at least 1010 liver cells are need to support bioartifical system that contain working liver cells. These liver cells first are drawn from rabbits. Then from pigs and finally from human cells.

The first successful bioartificial liver devices developed were filtered base mechanical detoxifying systems. These systems use a charcoal filter and a modified kidney dialysis machine to detoxify the blood may provide bridge to transplantation for patients awaiting organ donation or some patients obviate liver transplantation altogether.

BAL consists of a bioreactor, as single use plasma circuit and a machine to control the fluid flow through these components. Oxygen consumption rate is important parameter in the function of bioartificial liver. BAL compromises an extracorporeal process for continuously withdrawing a patient’s whole blood maintaining temperature, adjusting pH and perusing a hollow fiber bioreactor. It incorporates a biological and synthetic component coupled in such way to facilate the delivery of essential liver functions.

The goal of Bioartificial liver is to allow an auxiliary liver until native liver regeneration. The hepatocyted could allogenic or xenogenic animal origin. Large animal source of liver cells should allow immediate of fresh hepatocytes when BAL is required.

Used to remove toxins from the blood, capacity to maintain multiple liver functions such as protein synthesis, enzyme activity and drug metabolism, replace lost liver functions.

REQUIREMENTS FOR A LIVER ASSIST DEVICE/BIOARTIFICIAL LIVER
The FDA has a number of requirements that mainly deal with patient advocacy and safety.
If a cellular component is used, it must be purified and every component in it must be clearly identified.

The cellular preparation must be clearly shown to not transmit any infections diseases of any kind.

In terms of efficacy the cellular component must stay viable and active and must provide a steady amount of therapy to the patient.

If the device contains a synthetic component this component must be fully biocompatible.

The integrity of the material and parts must also be demonstrated such that the device won’t break down (causing the fire) or release particulate material into the blood of the patient.

The controlling mechanism (whether software based or mechanical) must also exhibit reliability.

In addition to the FDA’S requirement the device must also be 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.

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(7) In addition to the FDA’S requirement the device must also be 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.

(8) The patient’s circulation must interact with the treatment mechanism in a very specific manner the patient immune responses must be held in check or the device will be ruined by complement and coagulation mechanism.

### Enabling Technologies for Bioartificial Liver Development

**Hemodialysis/hemofiltration Hollow Fibers**

It is necessary for the controlled interaction of cells and circulating fluids. Biomaterials technology is also key to this area many devices are composed of a cell population surrounding an arrangement of hollow fibers. The fibers themselves and the material surrounding the fibers and cell population must both be biocompatible.

Cells used for liver therapy must be able to survive and/or proliferate in the device, and they must also maintain their specific liver function. Additionally, the activity of the cells themselves should not introduce dangerous materials into the body.

Cells can be isolated from liver tissue by digesting the extra cellular matrix and proliferating the cells in vitro. Cell therapy can then be used to induce the cells to become immortal.

### Cellular Components of Bioartificial Liver

#### Table 1

<table>
<thead>
<tr>
<th>Concept</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Hepatocytes</td>
<td>Cells isolated directly from the liver</td>
<td>exhibit specific functions</td>
</tr>
<tr>
<td>Immortalized Cell Lines</td>
<td>Immortalized hepatocyte lines that are obtained from cultures transforming human hepatocytes-genetically engineered to proliferate</td>
<td>-cells allow for longer device operating time than primary hepatocytes-if cultured from humans, it produces human proteins and cells are readily available</td>
</tr>
</tbody>
</table>

Hepatocytes must be anchored to a substrate in order to function properly.

**Anchoring methods for cells:** Various anchoring methods for cells are Micro carriers, Encapsulation, Hollow fibers.

Membrane area, pore size, and thickness can also affect biocompatibility and bioadhesion.
Different types of membranes used in BAL:

Different types of membranes used in BAL are Cellulose acetate, Cuprophan, Hemophan, Polyamide, Polypropylene, Polysulfone.

Current Work on the Bioartificial Liver

There are a number of companies currently studying different forms of the Bioartificial Liver. Described below are the major devices and studies involving

Table 2
Anchoring Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro carriers</td>
<td>Polymeric particles containing cells alginate micro spheres can be use</td>
<td>Can be combine with ECM material to improve</td>
</tr>
<tr>
<td>Encapsulation</td>
<td>Envelopment of hepatocytes in a polymeric matrix can use polysaccharides</td>
<td>Can be helpful in maintaining three dimension structure for hepatocytes</td>
</tr>
<tr>
<td>Hollow fibers</td>
<td>Luminal membranes that provide encourage for hepatocytes may be supplemented by ECM material i.e. collagen</td>
<td>May be coiled bundled or wound to allow maximum length surface area.</td>
</tr>
</tbody>
</table>

Table 3
Different Types of Membranes used in BAL

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Charge</th>
<th>MWCO (Kda)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose acetate</td>
<td>Positive</td>
<td>70-100</td>
<td>- Weak cell attachment. Sufficient metabolic activity. Low cell damage</td>
</tr>
<tr>
<td>Cuprophan</td>
<td>Neutral</td>
<td>&lt;12</td>
<td>- Low thrombogenicity- No considerable cell attachment- High percentage of damaged cells</td>
</tr>
<tr>
<td>Hemophan</td>
<td>Positive</td>
<td>&lt;10</td>
<td>- Adequate cell adhesion- Sufficient metabolic activity</td>
</tr>
<tr>
<td>Polyamide</td>
<td>Not Applicable</td>
<td>&lt;1000</td>
<td>- Adequate cell adhesion. Satisfactory biocompatibility</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>—</td>
<td>&lt;1000</td>
<td>- Adequate cell adhesion</td>
</tr>
<tr>
<td>Polysulfone</td>
<td>Negative</td>
<td>100</td>
<td>- Increased cell attachment. Low cell damage-Maintenance of metabolic activity</td>
</tr>
</tbody>
</table>

Table 4
Current Work

<table>
<thead>
<tr>
<th>Device</th>
<th>Company/School/ Organization</th>
<th>Clinical Phase</th>
<th>Design</th>
<th>Cell Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Dialysis UnitTM (formerly BioLogic-DT)</td>
<td>HemoTherapies (formerly HemoCleanse)</td>
<td>FDA approved</td>
<td>Membrane Separated</td>
<td>Noncellular (Charcoal)</td>
</tr>
<tr>
<td>Molecular Adsorbent Recycling System (MARS®)</td>
<td>Teraklin</td>
<td>I/II/II CE-approved</td>
<td>Hollow Fiber Bioreactor</td>
<td>Human Albumin</td>
</tr>
<tr>
<td>Extracorporeal Liver Assist Device (ELAD®)</td>
<td>Vitagen</td>
<td>I/II Multicenter</td>
<td>Hollow Fiber Bioreactor</td>
<td>Immortalized Human Hepatocytes</td>
</tr>
<tr>
<td>HepatAssist 2000 System</td>
<td>Circe Biomedical</td>
<td>III</td>
<td>Hollow Fiber Bioreactor</td>
<td>Porcine Hepatocytes</td>
</tr>
<tr>
<td>Bioartificial Liver Support Excorp Medical, Inc.</td>
<td></td>
<td>I/II Multicenter</td>
<td>Hollow Fiber Bioreactor</td>
<td>Primary Porcine Hepatocytes</td>
</tr>
<tr>
<td>LIVERX2000 System</td>
<td>Algenix, Inc.</td>
<td>I Center</td>
<td>Membrane Bioreactor</td>
<td>Primary Porcine Hepatocytes</td>
</tr>
<tr>
<td>Modular Extracorporeal Liver System (MELS®)</td>
<td>Charite Virchow</td>
<td>I/II Multicenter</td>
<td>Hollow Fiber Bioreactor</td>
<td>Human Hepatocytes</td>
</tr>
</tbody>
</table>
clinical trials. Below is a table summarizing current devices in clinical trials.

(1) Liver Dialysis Unit™ (HemoTherapies)
Formerly BioLogic-DT (HemoCleanse)

Figure 2: Liver Dialysis Unit™

Mechanical Design
The Liver Dialysis Unit is similar to kidney dialysis, but upon closer inspection, several differences become apparent. In the dialyzer, the blood is passed through a chamber instead of through much small tubes. The membrane separating the blood from the dialysate functions like a diaphragm, pumping the blood and dialysate in and out of the dialyzer alternately resulting in an average transmembrane pressure of 100-200 mm Hg. The volume of blood within the circuit varies from 200-250 cc depending on whether the system is in inflow or outflow.

Instead of a buffered aqueous solution, the Liver Dialysis Unit uses a mixture of sorbents including powdered activated charcoal, cation exchange resin, salts, a buffering agent, and macromolecular wetting substances. As in kidney dialysis, substances such as glucose and electrolytes can be added to the dialysate to prevent their removal from the patient’s blood.

The Process
From the patient perspective, the setup is similar to that for kidney dialysis. A catheter is inserted into a large vein, and then some blood is removed, passed through the dialyzer where toxins are removed, and returned to the patient through the same catheter. This process continues for 4-6 hours, and is repeated if needed.

(2) MARS® - Molecular Adsorbent Recycling System (Teraklin)

Figure 3: MARS Circuit

Mechanical Design
In Teraklin’s MARS system, blood is cleansed in an extracorporeal circuit that is a combination of both kidney and liver dialysis. Established methods for kidney dialysis do not work for liver failure because kidney dialysis removes only water-soluble toxins, while the liver normally removes albumin bound toxins. Albumin is a protein found in the blood that carries water insoluble substances including toxins. For this reason, MARS uses human albumin to cleanse the blood because it can attract toxins bound to albumin in the blood that the aqueous solution in kidney dialysis cannot remove. The system replaces the detoxification function of the liver.

The Process
The patient’s blood is passed through small tubes in a hollow fiber membrane hemodialyzer. On the outside of the tubes flows clean human albumin that acts as a dialysate. As the patient’s blood moves along the tubed, water-soluble and protein bound toxins in the blood are transported through the membrane and into the dialysate albumin on the other side. The membrane is impermeable to albumin and to other valuable proteins such as hormones and clotting factors, keeping them in the patient’s circulation. The cleaned blood then returns to the patient. Meanwhile, the albumin solution is recycled by passing first through a dialyzer opposite a buffered aqueous solution. This process is similar to that found in kidney dialysis and removes water-soluble substances...
from the albumin. The albumin then passes through an activated carbon adsorber and an anion exchanger that cleans it of albumin bound toxins.

Among the toxins that MARS can remove are Bilirubin, bile acids, Phenols, Mercaptans, Dioxin-like substances, Tryptophan, Ammonia, Copper, iron.

**Flow Rates**

<table>
<thead>
<tr>
<th>Blood Flow (ml/min)</th>
<th>Albumin Flow (ml/min)</th>
<th>Dialysate Flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No renal dysfunction</td>
<td>150–250</td>
<td>250</td>
</tr>
<tr>
<td>Additional renal dysfunction</td>
<td>150–250</td>
<td>250</td>
</tr>
</tbody>
</table>

**Benefits of the System Include**

1. Effective removal of protein bound and water soluble toxins.
2. Mimics the biological detoxification process of hepatocytes.
4. Control of glucose and lactate level.
5. Cost effectiveness due to recycling of toxin binding proteins.
6. High biocompatibility, high selectivity, cell-free operation.
7. Compatible with standard dialysis equipment.

**Excorp Medical, Inc.’s Bioartificial Liver Support System (BLSS)**

**Mechanical Design**

Excorp Medical, Inc. (Minneapolis) and the University of Pittsburg have collaborated to develop the 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 (raised by Midwest Research Swine). 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 Process**

The treatment lasts for approximately 12 hours. 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.

(5) Circe Biomedical’s HepatAssist 2000 System

**Mechanical Design**

The HepatAssist functions as an extracorporeal cell-based bioartificial liver device, relying on an open membrane hollow fiber bioreactor and porcine hepatocytes. These substances are exchanged between the hepatocytes – housed outside the hollow fibers – 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. Additionally, the device must be used in conjunction with a commercially available plasma separation machine, a heater, and temperature and oxygen monitors.

**The Process**

Each treatment generally lasts approximately six hours. The patients’ blood is first separated into plasma and cellular components in a plasmapheresis device. The cellular component remains in the plasmapheresis device, while the plasma goes through processing in the bioreactor. The plasma first goes through two charcoal filters, which filter out of the plasma massive bacteria and particulate matter that the system’s hepatocytes might be unable to handle. After this first detoxificative action, the plasma runs through the hepatocyte-lined hollow fiber column, which is the truly novel therapy that this device offers. The newly cleaned plasma is then reunited with the plasma component, which had been stored in the plasmapheresis device, and the whole blood is reinfused into the patient. During the process, a membrane oxygenator and heater are housed between the charcoal filters and hepatocyte bioreactor, with the purpose of keeping the plasma and the hepatocytes over which they flow at body temperature. The membrane oxygenator provides the porcine hepatocytes with the requisite oxygen for correct function.

(6) Algenix Inc.’s LIVERx2000 System

**Mechanical Design**

The LIVERx2000 device is a hollow fiber cartridge similar to that used for kidney dialysis. Primary porcine hepatocytes harvested from American-Yorkshire pigs that weight between 7 and 20 kilograms are suspended in a cold collagen solution and injected inside the fibers. The cartridge is then connected to the tubing circuit, and warm medium is perfused outside the fibers. The collagen begins to gel once it is warmed, and within 24hrs. The liver cells pull on the collagen gel to contract to 60% of its original diameter. In the resulting space, a nutrient-rich medium stream is perfused for normal functioning of the liver cells. At this point the device is ready to be hooked up to a patient. The patient’s blood is circulated outside the hollow fibers. The fiber membranes allow toxins from the blood to diffuse to the cells, but prevent immune molecules from reaching the cells. Two hollow fiber cartridges, each containing approximately 40 g of cells, are connected in series within an incubator maintained at 37 °C. Because the patient’s blood is taken from a vein, it is oxygenated to maintain a sufficient amount of oxygen for the hepatocytes. Temperature, pH and dissolved oxygen are all monitored on-line. The rest of the system consists of the necessary tubing and pumps. Heparin, an anticoagulation drug, is given to patients.

**The Process**

The LIVERx2000 is designed to treat both acute and chronic liver failure. With LIVERx2000, Algenix aims to increase the survival rate prior to transplantation, increase the survival rate after transplantation, serve as a bridge to liver regeneration in acute liver failure, enable more aggressive surgical removal of liver tissue in cancer and trauma patients, and reduce health care costs. The LIVERx2000 can function as a bridge to transplantation as well as a temporary liver support system as the liver regenerates and heals. The patient treatment time with the LIVERx2000 is eight hours for a maximum of two treatments.

(7) Modular Extracorporeal Liver System (MELS)

**Figure 5: Flow Chart Diagram of Clearance of Blood by MELS**
Mechanical Design

The Charité Virchow Clinic in Berlin under Igor M. Sauer has designed the Modular Extracorporeal Liver System (MELS). Between a network of thin hollow fiber membranes, human hepatocytes or liver cells are kept alive by a constant supply of oxygen and a “culture medium” to feed on. The modular design is based on parallel plate geometry. Each functional element consists of a rectangular cross-section flow channel formed by two polycarbonate plates. The lower plate supports a semi-permeable membrane to which the liver cells are attached. Parallel arrays of gas permeable hollow fibers (200 mm ID x 280 mm OD) are mounted on the upper plate. Blood plasma from the patient flows along the channel and is therefore in direct contact with the liver cells. The modular design allows for scale-up of the device; a 10 channel stack contains 2.5 x 10^8 cells (500 grams). The cells can survive for up to two months in these conditions. The flow rate through the bioreactor is 100 mL/min.

The Process

Patients’ plasma is separated from their blood in a plasma separator before the plasma is allowed to pass into the bioreactor (filtration plasmapheresis). As it is flowing over the capillary-like network of fibers, the liver cells function normally, drawing toxins out of the plasma, effectively cleaning and refreshing the patient’s blood without actually mixing with it. Heparin, an anticoagulation drug, is delivered to patients.

A Life-Saving Liver Machine

The plasma and smaller blood cells continue on their circuit into a tube immersed in the liquid suspension of clustered pig cells. The pore size of the tube’s membrane allows blood to flow in and out of the hollow fiber while the hepatocytes remove bile, ammonia, urea, and other impurities. The pore size also blocks the hepatocytes and any pig cell debris from entering the patient’s blood.

Working

(1) Blood from the patient first courses through membranes in a “hollow fiber cartridge” that separate red cells and plasma from the larger white blood cells.

(2) The plasma and smaller blood cells then pass through a tube called a “settling column” immersed in the liquid suspension containing clusters of pig liver cells, or “porcine hepatocyte spheroids” kept alive in an oxygenated solution.

(3) The tube’s membrane allows blood to flow in and out to be cleansed by the hepatocytes, but blocks pig tissue from entering the patient’s blood.

The cleansed blood is then reunited with the white blood cells and returned to the patient’s body.

Challenges for Bioartificial Liver

There are many reasons due to which the development of BAL is very slow.

(1) For the limited progress in the assessment of artificial-liver systems is the plethora of options and variables to be tested, which include use of hepatocytes or not, choice of cell-source and cell-line, bioreactor design, incorporation of filtering and charcoal columns, and which patients to study and for how long.

(2) It include the use of non-human cells and the possible transmission of virus from animals to humans, and more fundamentally, the vast number of functions performed by the liver, some of which are yet to be completely understood. Currently, human trials have been limited to extracorporeal devices that filter the blood much like hemodialysis machines. If this work progresses, we may see even more similarities between the treatment of liver failure and kidney failure.

(3) Proteins larger than the pore size of the device cannot be released by the hepatocytes, limiting the efficacy of the device.
(4) One major hurdle to implementing this therapy in humans is the size requirements of the device.

(5) The device is very large and not used in common cases.

CONCLUSION

The bioartificial liver improves cerebral function, and may be used as bridge to transplantation with acute or acute-on-chronic liver failure to liver transplantation or recovery. Generally it’s safe and lead to improvement in lab. Parameters and clinical conditions such as hepatic encephalopathy.

It is field of tissue engineering is the effective reutilization of living cells in man made housing to effectively reproduce organ function. Devices known as bioreactor or bioartificial constructs are highly dependent upon selection of effective fabrication geometrics, such that all cells within the device receive adequate nutrients and oxygen. A fundamental understanding of the cues that promote phenotypic stability and tissue morphogenesis will undoubtedly contribute to next generation of extracorporeal devices, cell transplantation therapies, and tissue engineering constructs.

References


