Liver Transplantation and Biotechnology

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Introduction

The liver is a vital organ in the human body, crucial to maintaining overall health and homeostasis. Its many functions are crucial for the detoxification of harmful substances, regulation of metabolic processes, and maintenance of various physiological systems. Understanding the liver's functions and the consequences of its failure is essential for comprehending the role it plays in health and disease.

Importance of the Liver in Human Physiology

The liver is often referred to as the body's biochemical factory due to its extensive array of functions. It plays a critical role in regulating metabolism, producing essential proteins, and facilitating digestion through bile production. The liver's functions are integral to maintaining homeostasis, ensuring that various biochemical processes are balanced, and that the body operates efficiently. Its health is paramount for overall well-being, and any compromise in its function can lead to significant health issues. Currently, liver disease accounts for two million deaths annually, and is responsible for 4% of all deaths[4].

Liver Function

Functions of the Liver

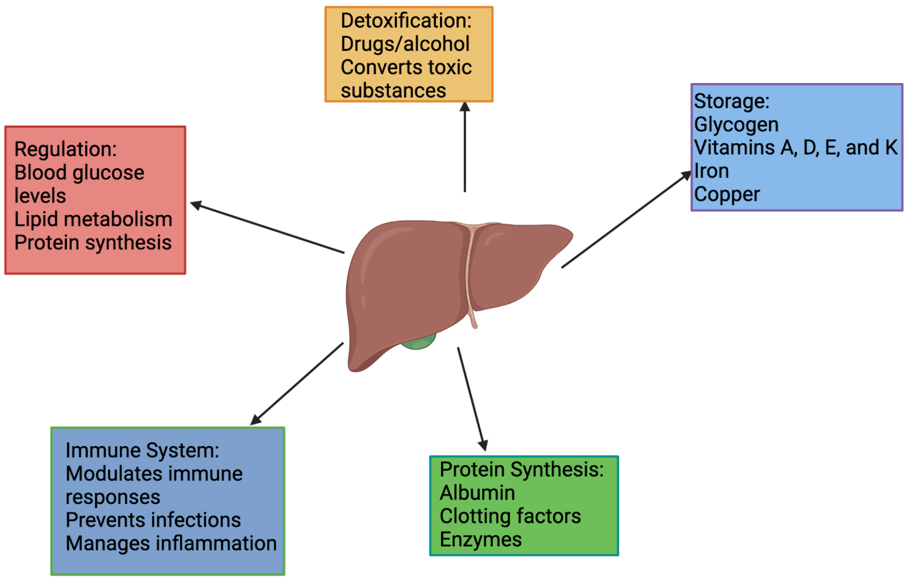

Figure 1: The liver has many functions, including the detoxification of toxic substances, regulating the blood composition, modulating our immune responses, synthesizing proteins, and storing important vitamins and nutrients.

Detoxification

One of the liver's primary roles is detoxification by filtering blood coming from the digestive tractplainand removing toxins and metabolic waste products. It converts these substances into less harmful forms that can be excreted through bile or urine. The liver uses enzymatic processes to metabolize drugs, alcohol, and environmental toxins. This detoxification process is essential for preventing the accumulation of harmful substances–such as alcohol–in the body.

Immune Function

The liver also plays a role in the immune system through phagocytic cells, known as Kupffer cells, which clear pathogens and debris from the bloodstream. The liver helps to modulate immune responses and maintains a balance between immune tolerance and activation. This function is crucial for preventing infections and managing inflammation.

Synthesis

The liver is responsible for the synthesis of several important proteins, including albumin, clotting factors, and enzymes necessary for digestion. Albumin, a major plasma protein, helps maintain blood volume and pressure by regulating the oncotic pressure in blood vessels. The synthesis of clotting factors is crucial for proper blood coagulation and wound healing. Additionally, the liver produces bile acids from cholesterol, which are essential for the digestion and absorption of dietary fats.

Storage
The liver acts as a storage center for several vital nutrients and substances. It stores glucose in the form of glycogen, which can be converted back into glucose when needed to maintain blood sugar levels. Additionally, the liver stores vitamins and minerals, including vitamins A, D, E, and K, iron, and copper. This storage function helps to regulate nutrient availability and supports various metabolic processes.

Regulation of Blood Composition
The liver is integral to regulating blood composition. It helps to regulate blood glucose levels, lipid metabolism, and protein synthesis. The liver also contributes to the breakdown and recycling of red blood cells, managing the balance of various blood components, and ensuring the efficient removal of waste products.

Liver Failure

Liver failure occurs when the liver is unable to perform its essential functions, leading to a range of systemic complications. Liver disease is the 11th leading cause of death worldwide. It can be classified into two main types: chronic liver failure and acute liver failure.

Chronic Liver Failure
Chronic liver failure, also known as chronic liver disease, develops over a prolonged period from several months to many years, and is often the result of ongoing liver damage from conditions such as chronic hepatitis and long-term alcohol abuse. Hepatitis is the inflammation of liver tissue, and there are five types of hepatitis, but the three most common are hepatitis A, B, and C. The gradual loss of liver function leads to a progressive decline in its ability to detoxify the blood, synthesize proteins, and regulate metabolism. Symptoms of chronic liver failure include jaundice (yellowish discoloration of the whites of the eyes, skin, and mucous membranes caused by deposition of bile salts in these tissues), ascites (abnormal buildup of fluid in the abdomen), and hepatic encephalopathy (brain dysfunction due to toxin buildup).
These symptoms develop over the four stages of liver disease-

- Stage 1: Inflammation
- Stage 2: Fibrosis/scarring
- Stage 3: Cirrhosis (scar tissue takes over and the liver stops working)
- Stage 4: End-stage liver failure.

Management typically involves addressing the underlying cause and may require liver transplantation in advanced cases. At stage four, only a liver transplant can cure it.[9]

Acute Liver Failure
Acute liver failure (ALF) is a sudden and rapid decline in liver function, which can occur within days or weeks. Causes include viral hepatitis, drug-induced liver injury (such as from

acetaminophen overdose), or exposure to toxins. Acute liver failure can lead to severe complications, including hepatic encephalopathy, coagulopathy, and multi-organ failure. Immediate medical intervention is crucial, and treatment often involves supportive care and sometimes emergency liver transplantation. In ALF, the adult mortality is approximately 50%, despite the increase in the number of patients receiving liver transplants.

Stages of Liver Failure

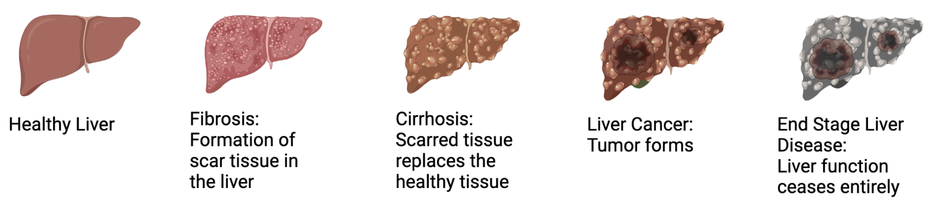

Figure 2: Chronic liver failure can take place over several months, to many years, while acute chronic liver failure can take place within days or weeks. Both result in the liver's function ceasing entirely.

Liver Transplantation

Key Developments in Liver Transplantation[5]

Brain Death Legislation
Through the establishment of "brain death" also known as an irreversible coma, the Brain Death Legislation allows the retrieval of organs from brain dead patients in near normal physiological conditions.

Drug Therapies for Immunosuppression
Immunosuppressants are crucial for preventing the body from rejecting the new liver. When a liver is transplanted, the recipient's immune system recognizes the new liver as foreign. This triggers an immune response aimed at attacking and destroying the transplanted organ. 30% percent of patients reject their transplanted liver, and the risk is highest during the first six

months. Immunosuppressants are used to dampen this immune response and prevent rejection. The following are different types of immunosuppressants with different mechanisms:

Calcineurin Inhibitors - These drugs block a protein that activates T-cells (white blood cells that help your immune system fight germs and disease), which helps reduce the immune system's ability to attack the transplanted liver.

Antimetabolites - Antimetabolites interfere with DNA production in immune cells, limiting their growth and proliferation to help prevent liver rejection.

mTOR Inhibitors - Sirolimus and Everolimus inhibit a protein involved in cell growth and division, which helps reduce the activity of immune cells attacking the liver.

Corticosteroids - Prednisone and similar drugs have broad anti-inflammatory effects, reducing overall immune activity and inflammation, often used to manage acute rejection episodes.

Biologics and Monoclonal Antibodies - These agents target specific molecules or cells involved in the immune response, helping to prevent rejection by blocking key immune system components.

These immunosuppressants work together to prevent the body from rejecting the liver while also managing any potential side effects.

Organ Preservation Techniques
In 1987, a major advancement in managing ischaemia-reperfusion syndrome (the tissue damage caused when blood supply returns to tissue after a period of ischemia or lack of oxygen) in donor organs and mitigating cellular swelling was achieved. The development of a groundbreaking organ preservation fluid by surgeon Folkert Belzer significantly expanded the time that livers could be preserved outside the body, increasing the period from under 8 hours to over 15 hours. This extension made it feasible to transport donor livers over longer distances and alleviated some of the urgency typically associated with the transplantation process.

Coagulation Control
Early liver transplantation procedures often resulted in substantial blood loss, leading to significant morbidity and mortality. The risk of bleeding during liver transplantation is influenced by multiple factors and necessitates frequent use of blood and blood products, including fresh frozen plasma, clotting factor concentrates, and platelets (tiny blood cells that help a body form clots to stop bleeding). Contributing factors to bleeding include the severity of existing liver disease, portal hypertension (increased blood pressure in the portal vein, which carries blood from the digestive organs to the liver), varices (abnormally dilated veins, often found in the esophagus or stomach, that can occur due to portal hypertension), abdominal adhesions, and pre-operative blood counts. Blood loss during a transplant of ≥31.25 mL/kg can also predict postoperative thrombosis (the formation of a blood clot inside a blood vessel, obstructing the flow of blood through the circulatory system.)

With the growth of liver transplantation programs, the demand for blood services has increased. Excessive blood transfusions can negatively impact clinical outcomes, such as hospital stay duration and survival rates for both patients and grafts. Efforts to minimize transfusion needs have included lowering central venous pressure, using various blood products (such as fresh frozen plasma, cryoprecipitate, and platelets), employing haemodilution (a dilution of blood constituents by plasma replacement or blood removal), and performing intraoperative blood salvage autotransfusion (recovering blood lost during surgery and re-infusing it into the patient). Thanks to these improvements, it is now possible for many liver transplants to be completed without the need for blood transfusion.

Donor After Brain Death (DBD) and Donor After Circulatory Death (DCD)
Before the Harvard criteria for brain death was established in 1968, liver transplants depended on non-heart-beating donors (NHBDs), making all such transplants donated after cardiac death (DCD). From 1968 until the 1990s, most liver transplants were performed using organs from donated after brain death (DBD) donors. In 1995 the 'Maastricht classification' for organ donation following circulatory arrest was developed, which includes five categories:

Category 1: arrived already deceased at the hospital

Category 2: unsuccessful resuscitation

Category 3: life-sustaining treatment withdrawn due to the futility of further care

Category 4: cardiac death after brain death

Category 5: unexpected arrest while in intensive care

Categories 3 and 4 are classified as controlled circulatory arrest, where the retrieved organs are less likely to suffer from ischemic injury complications.

Living Donor Liver Transplantation
Despite the positive impact of brain death criteria on organ transplant outcomes in Western countries, this approach did not gain cultural acceptance in many Middle Eastern and Asian nations, such as Japan and Korea, even with laws supporting brain death. Consequently, the shortage of organ donors continued to be a significant obstacle to transplantation in Asia. However, the field of transplantation in Asia saw substantial progress with the introduction of a technique by Henri Bismuth from France, who developed a method to reduce an entire adult liver to just the left lobe for implantation in a child. Building on this success, Rudolf Pichlmayr from Hannover, Germany, advanced the field in 1988 with the split-liver transplant, which involved dividing a single liver allograft for use in two separate recipients. This innovation paved the way for partial hepatectomy, where a portion of the liver is removed from a living donor and transplanted into a recipient with liver disease.

Living donor liver transplantation emerged as a crucial solution to the donor shortage, utilizing a range of liver graft types, including full right, left, and left lateral grafts, as well as various partial grafts such as right anterior, right posterior, and extended lobe grafts.
Living donors, who are typically healthy individuals with no medical benefit from the procedure, require careful consideration to minimize morbidity and mortality. Pre-operative evaluations include imaging to identify abnormal vascular and biliary anatomy, liver fat assessment via MRI or biopsy, and liver volumetry, which involves calculating the estimated remnant liver volume and graft-to-recipient weight ratio (GRWR) to mitigate small-for-size issues for both donor and recipient.

Hepatocyte Transplant
Human hepatocyte transplantation, introduced in Japan in the early 1990s, serves as an alternative to whole-organ liver transplants. Hepatocytes, the primary functional cells of the liver, can be repeatedly transplanted and utilized across multiple patients from a single donor organ. This method benefits from the ability to cryopreserve hepatocytes, making them available for future use. Transplanted hepatocytes can support the function of the native liver, allowing it to remain in place and continue providing some liver functions even if the donor hepatocyte graft fails. This preservation of the native liver also offers potential for future treatments, especially for metabolic liver disorders.

Initially, hepatocyte transplantation involved autologous cells harvested from the left lateral segments of the liver in a cohort of ten patients with liver cirrhosis, aiming to provide metabolic support. The hepatocytes were delivered through various methods, including direct splenic puncture, portal vein, and splenic artery injections.

Liver Transplantation Limitations

Liver transplantation, while a crucial treatment for patients with severe liver disease, has many significant limitations. The main challenge is the shortage of donor organs, which often results in long waiting times and worsens patient outcomes as they wait for a suitable liver. Additionally, not all donated livers are viable for transplantation due to issues like donor age or organ quality. The procedure itself carries risks, including potential complications from surgery, such as bleeding and infection. Post-surgery, recipients face the risk of organ rejection, which can be prevented by immunosuppression drugs, but may require the patients to maintain a lifelong use of the drugs. These drugs, while preventing rejection, increase the risk of infections and have many side effects. Managing these medications requires strict adherence, as non-compliance can lead to serious complications or graft failure.[5]

Long-term outcomes can be problematic, with issues such as chronic rejection and graft failure over time. Patients must also navigate pre- and post-transplant challenges, including maintaining overall health before surgery and managing ongoing care afterward. Socioeconomic and geographic factors can further affect access to transplantation services and post-operative care, leading to disparities in outcomes.

Ethical concerns also arise, particularly regarding the allocation of limited donor organs. The process involves complex decisions about who should receive a transplant based on factors like medical urgency and potential for success. Living donor transplants offer an alternative but introduce risks for the donor, including potential complications and long-term health impacts. Addressing these limitations requires continued research, better organ donation systems, and improved medical strategies to enhance both short-term and long-term outcomes for liver transplant recipients.

Bioartificial Liver Technology

Artificial Organs

Artificial organs can be broadly categorized into three main classes based on their composition and functionality: mechanical, biomechanical, and biological (bioartificial) organs.[8]

Mechanical artificial organs are made entirely from non-biological materials such as metals, plastics, and ceramics. These devices function through engineering principles and mechanical systems to replace or support the functions of failing biological organs. Examples include artificial hearts like the Total Artificial Heart (TAH), which uses mechanical pumps to replace the heart's pumping action, and dialysis machines that filter blood to perform the functions of the kidneys. Prosthetic limbs, while not organs, fall into this category due to their mechanical nature and function in replacing or augmenting lost limbs.

Biomechanical artificial organs combine living cells with non-living materials, integrating biological components with mechanical systems to enhance functionality and biocompatibility. These organs use synthetic polymers, metals, and biological cells to create a hybrid system. For instance, bioengineered skin used for grafting includes living skin cells grown on synthetic scaffolds, and cell-seeded scaffolds in tissue engineering create functional tissue structures. The hybrid artificial pancreas combines mechanical insulin delivery systems with biological sensors to regulate blood glucose levels more effectively.

Biological (bioartificial) artificial organs consist primarily of living cells, often supported by biodegradable polymers and sometimes metal components. These organs are designed to closely mimic the functions of natural organs by creating a biological environment that replicates natural organ functionality. Examples include bioartificial livers containing hepatocytes, which are liver cells supported by a system using biodegradable materials, and tissue-engineered organs like bladders or tracheas grown from a patient’s own cells. These organs aim to restore natural functions as closely as possible, offering potential for better integration and functionality within the body. This paper is primarily focused on bioartificial organs.

The Molecular Adsorbent Recirculating System (MARS)

The Molecular Adsorbent Recirculating System (MARS) is a specialized technology designed to temporarily support patients with acute liver failure or severe chronic liver disease. It operates on principles similar to dialysis but is tailored specifically for liver function, making it a crucial component in bioartificial liver technology.

MARS works by filtering the patient's blood through a sophisticated system that uses a combination of adsorption and dialysis to remove toxins. Blood is drawn from the patient and passed through a circuit that includes a special membrane and adsorbent columns. The membrane separates the blood from a dialysate fluid, while the adsorbent columns bind and remove specific toxins, such as bilirubin and other metabolic waste products that the liver would normally process. In addition to adsorption, the system performs dialysis, where the blood passes through a semi-permeable membrane to remove smaller, water-soluble toxins and excess water. The cleaned blood is then returned to the patient's body.[3]

In the context of bioartificial liver technology, MARS plays a significant role as a temporary solution. It provides essential liver function support by detoxifying the blood and managing a broader range of toxins compared to traditional dialysis. This capability is particularly valuable for patients awaiting liver transplants or those undergoing treatments involving bioengineered liver cells. MARS can stabilize patients while they wait for a suitable transplant or allow their liver to recover from acute failure.

Overall, MARS is a sophisticated bridging therapy that supports liver function and improves patient outcomes during critical periods. Its advanced filtration techniques make it a vital tool in managing liver failure and enhancing the efficacy of other liver treatments.

MARS (Molecular Adsorbent Recirculating System)

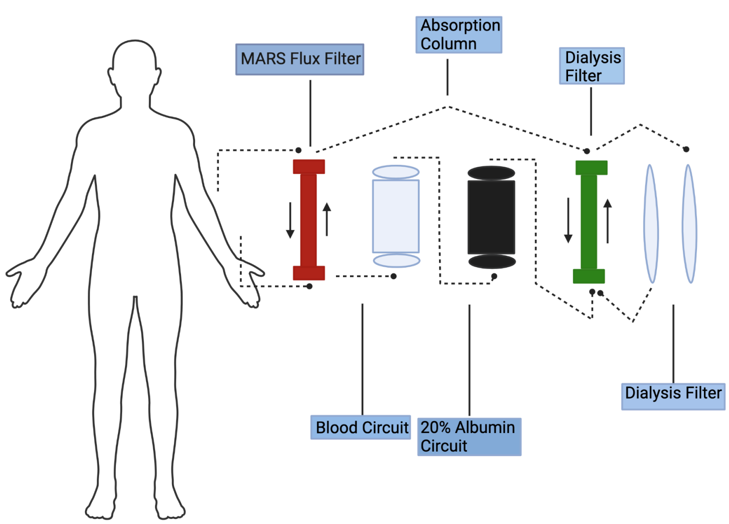

Figure 3: MARS supports patients with liver disease by filtering the blood and detoxifying it, acting as a temporary replacement for the liver.

Single-Pass Albumin Dialysis (SPAD)

Single-Pass Albumin Dialysis (SPAD) is a specialized blood purification technique designed to address the removal of toxins bound to albumin, a key protein in blood plasma. Unlike traditional dialysis methods that primarily target small molecules through diffusion, SPAD focuses on larger, albumin-bound toxins. In this process, blood is drawn from the patient and passed through a dialyzer—a device that separates the blood into two compartments. Inside the dialyzer, the blood encounters an albumin-based solution, which binds to these toxins. The blood, now cleared of these harmful substances, is then returned to the patient in a single pass through the dialyzer, making the procedure efficient and straightforward.[1]

In the realm of bioartificial liver technology, SPAD plays a crucial role by enhancing the detoxification capabilities of these devices. Bioartificial livers are designed to replicate or support liver functions, and integrating SPAD can significantly improve their performance. By targeting toxins that are specifically bound to albumin, SPAD complements other detoxification methods used in bioartificial livers, helping to ensure that a broader range of harmful substances is removed from the blood.

The integration of SPAD into bioartificial liver systems offers several benefits. For patients suffering from acute liver failure or those awaiting liver transplants, SPAD can provide critical support by alleviating the burden on any remaining liver tissue or bioartificial components. Additionally, SPAD can be used to monitor blood composition and toxin levels in real time, allowing for adjustments to the dialysis process and optimizing the overall efficacy of the bioartificial liver.

MARS vs. SPAD

	MARS (Molecular Adsorbent Recirculating System)	SPAD (Single Pass Albumin Dialysis)
What do they do, and how do they do it?	MARS filters the patient's blood through a system that uses a combination of adsorption and dialysis to remove toxins.	SPAD addresses the removal of toxins bound to the key protein, albumin, by filtering blood through a dialyzer, a device that separates the blood into two compartments.
Benefits	● MARS provides essential liver support ● Detoxifies blood ● Broader toxin management than dialysis ● Key for patients awaiting transplants or bioengineered liver cells ● Stabilizes while waiting for transplant or liver recovery	● SPAD alleviates the burden on any remaining liver tissue or bioartificial components. ● It can be used to monitor blood composition and toxin levels in real time

Disadvantages	• Energy consumption • Complexity of design • Maintenance Requirement • Initial Costs	• Limited efficiency • Short treatment time • Cost Complexity • Potential for instability • Limited patient suitability
In what ways are they different?	MARS can be a temporary solution, mimicking the liver's detoxification function.	SPAD is a specialized blood purification technique that enhances the detoxification function of other bioartificial liver devices.

Bioartificial Liver and Liver Transplant Comparison

Liver Transplantation can significantly improve a patient's quality of life and overall health. When performed at specialized centers, liver transplants have high success rates, and many patients experience long-term survival and can return to their normal activities. However, one of the major drawbacks is the critical shortage of donor organs. This scarcity leads to long waiting times, and patients often face a high risk of mortality while awaiting a suitable match. The surgical procedure itself is complex and involves risks such as infections, bleeding, and complications related to anesthesia.

Post-transplant, patients must adhere to a regimen of immunosuppressive medications for life to prevent the rejection of the new liver. These medications come with their own set of challenges, including side effects and an increased risk of infections and certain types of cancer. Additionally, there is always a risk of acute or chronic rejection of the transplanted liver, which may necessitate further treatments or interventions. Despite these challenges, liver transplantation remains a well-established treatment with a track record of success for many patients.
In contrast, bioartificial livers are an emerging technology designed to address liver failure. They offer potential as a bridging therapy, stabilizing patients while they await a donor organ. If developed and deployed effectively, bioartificial livers could alleviate some of the pressure on

the organ transplant system by reducing the dependence on donor organs. Many of these devices are less invasive than surgical options and could be used temporarily to support liver function.

However, bioartificial livers are still largely experimental and may not yet be widely available or approved for routine clinical use. They currently serve as a short-term solution rather than a permanent replacement, and many do not fully replicate all the functions of a natural liver. Technical and biological challenges persist in creating devices that can effectively mimic liver functions and integrate seamlessly with the human body. Furthermore, while bioartificial livers show promise, their long-term impacts and potential side effects are still not fully understood. In summary, liver transplantation provides a potential cure for liver disease but is limited by donor organ shortages and the complexities of surgery and long-term medication.

Bioartificial livers, while promising as a bridge to transplantation and a potential solution to organ shortages, are still in development and face significant technical and biological challenges. The choice between these options depends on various factors, including the availability of donor organs, patient needs, and advancements in bioengineering technology.

Where the Bioartificial Liver Field is Now

The bioartificial liver field has seen remarkable progress in recent years, driven by advancements in bioengineering and regenerative medicine. Researchers are actively developing various types of bioartificial liver devices that aim to support or replace liver function in patients with liver failure. These devices include those that use liver cell cultures, tissue engineering techniques, and even stem cells to mimic essential liver functions such as detoxification, protein synthesis, and metabolism.

Currently, several bioartificial liver devices are undergoing clinical trials to assess their safety and effectiveness. These trials are a critical step in determining how well these devices perform in real-world settings and in identifying any potential risks or complications. Clinical trials help to refine these technologies and ensure that they provide a viable solution for patients in need of liver support.

There are different types of bioartificial liver devices being explored. Hepatocyte-based devices use cultured liver cells to perform liver functions and can be designed as temporary support systems or more permanent solutions. Additionally, bioreactors are being developed that maintain liver cells in a controlled environment to enhance their function. Hybrid devices, which combine biological components with synthetic materials, aim to improve liver function support by integrating living cells with engineered structures.

Despite these advances, several challenges remain. Maintaining the viability and functionality of liver cells outside the body is a significant hurdle. Additionally, ensuring that bioartificial liver devices integrate seamlessly with the patient's body without causing adverse reactions is an ongoing concern. The regulatory pathways for the approval of these devices are complex and can be time-consuming, adding another layer of challenge to their development.

Looking ahead, research in the bioartificial liver field is focusing on improving the longevity and performance of these devices. Scientists are exploring new cell sources and enhancing the integration of these devices with the patient's physiology. There is also growing interest in combining bioartificial livers with other treatments, such as gene therapy or immunomodulation, to address liver diseases more comprehensively. While significant progress has been made, continued research and clinical testing are essential to overcome current limitations and bring these innovative solutions to broader clinical use.

Where the Bioartificial Liver Field Could go in the Future

The future of the bioartificial liver field is poised for exciting advancements, driven by ongoing research and technological innovation. One promising direction is the development of advanced cell technologies. Researchers are exploring the use of pluripotent stem cells or genetically engineered cells to create more functional and durable liver cells. This could enhance the performance of bioartificial liver devices, making them more effective in mimicking the complex functions of the liver and extending their longevity.

Another potential advancement lies in integrating bioartificial liver devices with regenerative medicine. By combining these devices with tissue engineering techniques, scientists aim to create bioengineered liver tissues or even complete organs. This integration could offer long-term solutions for liver failure, potentially reducing the dependence on organ transplants and providing a more sustainable approach to treating severe liver conditions.

Personalized medicine is also likely to play a significant role in the future of bioartificial liver technology. Advances in genomics and individualized treatment plans could lead to the development of bioartificial liver devices tailored to the specific genetic and physiological needs of each patient. Such customization could improve the effectiveness of these devices and minimize the risk of adverse reactions, offering more precise and effective treatments.

The field might also see the emergence of hybrid and smart devices that incorporate advanced materials and sensors. These devices could provide real-time monitoring and adjustment of liver functions, allowing for dynamic responses to the patient's changing needs. Such technological advancements could significantly improve the adaptability and functionality of bioartificial liver systems.

Looking further ahead, research may focus on developing bioartificial liver devices capable of providing long-term or permanent liver support. This could involve creating more durable and efficient devices that support liver function indefinitely, potentially offering a viable alternative to traditional liver transplants.

Finally, the integration of bioartificial liver devices with other therapeutic approaches, such as gene therapy or immunotherapy, could enhance overall treatment strategies for liver diseases. Combining these technologies might offer comprehensive solutions that address the underlying causes of liver failure and improve patient outcomes, pushing the boundaries of what is currently possible in liver disease management.

References

1. García Martínez JJ, Bendjelid K. Artificial liver support systems: what is new over the last decade? Ann Intensive Care. 2018 Nov 15;8(1):109. doi: 10.1186/s13613-018-0453-z. PMID: 30443736; PMCID: PMC6238018.
2. Kryou C, Leva V, Chatzipetrou M, Zergioti I. Bioprinting for Liver Transplantation. Bioengineering (Basel). 2019 Oct 10;6(4):95. doi: 10.3390/bioengineering6040095. PMID: 31658719; PMCID: PMC6956058.
3. Tandon R, Froghi S. Artificial liver support systems. J Gastroenterol Hepatol. 2021 May;36(5):1164-1179. doi: 10.1111/jgh.15255. Epub 2020 Oct 3. PMID: 32918840.
4. Global burden of liver disease: 2023 update
   Devarbhavi, Harshad et al.
   Journal of Hepatology, Volume 79, Issue 2, 516 - 537
5. Khalil A, Quaglia A, Gélat P, Saffari N, Rashidi H, Davidson B. New Developments and Challenges in Liver Transplantation. J Clin Med. 2023 Aug 27;12(17):5586. doi: 10.3390/jcm12175586. PMID: 37685652; PMCID: PMC10488676.
6. García Martínez, J.J., Bendjelid, K. Artificial liver support systems: what is new over the last decade?. *Ann. Intensive Care* 8, 109 (2018).
7. "Bioartificial Organ Manufacturing Technologies." Review by Xiaohong Wang. Sage Journals. SAGE Journals Online, dpl6hyzg28thp.cloudfront.net/media/10.1177_0963689718809918.pdf. Accessed 24 Sept. 2024.
8. R, Kushaal. The Development of Artificial Organs and Tissue Engineering. Microsoft Word, www.researchgate.net/profile/R-Aakash-Kashyap/publication/379256512_The_development_of_artificial_organs_and_tissue_engineering/links/660180a8d3a08551424f61ad/The-development-of-artificial-organs-and-tissue-engineering.pdf. Accessed 30 Sept. 2024.
9. Whitbourne, Kathryn. "What Is Liver Failure?" WebMD, 10 June 2024, www.webmd.com/digestive-disorders/digestive-diseases-liver-failure. Accessed 30 Sept. 2024.