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Cellular therapy and bioartificial approaches to liver replacement

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Abstract

Purpose of review—The success of liver transplantation has increased over the past 20 years due to improved immunosuppressive medications, surgical technique and donor-recipient selection. To date, the number of patients waiting for a liver transplant exceeds the number of transplants performed yearly by over a 2: 1 ratio. Despite efforts to expand the donor pool, mortality of patients waiting for a liver remains high due to the shortage of donor organs. Herein, we discuss options for liver replacement that are currently under development.

Recent findings—Extracorporeal bioactive liver perfusion devices were investigated in the late 1990s and preliminarily demonstrated safety but failed to show clinical efficacy. Current research is ongoing, but the focus has shifted to xenotransplantation of whole organs, organ engineering and cell transplantation. These new modalities are limited to small and large animal studies and each present unique advantages and limitations.

Summary—Discovery of new sources of organs or cells to replace a damaged liver may be the only long-term solution to provide definitive therapy to all patients who require transplantation. The past 2 years have seen notable achievements in xenotransplantation, tissue engineering and cell transplantation. Though challenges remain, now identified, they may be readily solved.

Keywords

cell transplantation; extracorporeal liver perfusion; liver transplantation alternatives; organ engineering; tissue engineering; xenotransplantation

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Conflicts of interest

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INTRODUCTION

Liver transplantation is the definitive therapy for acute and chronic liver failure, and the success of liver transplantation has now become one of its most significant obstacles [1]. The number of patients waiting for a liver transplant (>16 000 patients) far surpasses the supply of organs from standard criteria brain dead donors (6000–7000 liver donors annually), yet those who receive a liver transplant have improved 1-year, 3-year and 5-year graft survival compared with patients who were transplanted just 20 years ago.

The ability to engineer bioartificial liver replacement therapies remains in the investigatory stages, either as a bridge to definitive orthotopic liver transplantation or as destination therapy. This review summarizes the highlights in emerging strategies to develop liver substitutes. Extracorporeal liver perfusion systems have had limited success as a bridge to transplantation. Xenotransplantation, whole organ tissue engineering and liver repopulation using hepatocyte transplantation are new technologies in various stages of development with future implications to surgical care and management of end-stage liver disease.

EXTRACORPOREAL BIOACTIVE LIVER PERFUSION SYSTEMS

Extracorporeal liver perfusion systems are divided into nonbiologic hemofiltration devices that utilize activated charcoal, albumin or other concentration gradients to cleanse blood from patients with liver failure. For the purposes of this review, we will briefly focus on bioartificial liver support systems. These devices utilize hepatocytes from animal or human sources. The most developed systems are the HepatAssist and the Extracorporeal Liver Assist Device (ELAD). The HepatAssist uses porcine hepatocytes immobilized onto a semipermeable hollow fiber membrane that separates porcine cells from the patient's blood circuit. It was the first liver bioartificial assist device to be tested in a phase II/III clinical trial. One hundred and seventy one patients with fulminant or subfulminant liver failure or primary graft nonfunction after transplantation were evaluated at several centers and 85 underwent treatment with HepatAssist. Favorable safety was demonstrated but improved 30-day survival was not achieved in the overall study group. There is some indication, however, that patients treated with the bioartificial device had improved survival compared with controls receiving best medical therapy when outcomes were adjusted for patients who underwent liver transplantation [2]. Current research is ongoing to improve on the original version of the HepatAssist, including the use of an increased biomass of porcine hepatocytes.

ELADs use hollow fiber membranes in conjunction with human C3A hepatoblastoma cells placed into cartridges. Early, pilot clinical trials in the United States analyzed a small population (<24 patients) with acute liver failure. Those treated with the ELAD had equivalent survival compared with controls in cohorts with recoverable acute liver injury and patients with more severe hepatic deterioration requiring listing for liver transplant [3]. Taken together, bioartificial liver support systems have shown initial safety in small, pilot studies in the late 1990s and early 2000s but have not demonstrated a convincing survival benefit to date, though a few clinical trials have been initiated in the last few years. Potential benefits include a readily available supply of human cells lines or porcine cells. The possibility of disease transmission using porcine cells or induction of cancer using human hepatoblastoma cells within these systems remains to be thoroughly tested in controlled clinical trials.

XENOTRANSPLANTATION OF THE LIVER

Xenotransplantation offers an unlimited supply of donor organs for transplantation, and the majority of promising results have come from studies using porcine organs. The major

limitations to xenotransplantation are hyperacute and acute rejection of foreign porcine antigens presented on donor cells and concerns about transmission of endogenous retroviruses and other foreign organisms. Hyperacute rejection occurs within minutes to hours after implantation of xenogeneic grafts into primate recipients. This typically results from the binding of naturally occurring antibodies in the primate recipient with discrete epitopes on the vascular endothelium of xenogeneic grafts. The common target of these preformed antibodies are galactose-α(1,3)galactose, which is a terminal disaccharide synthesized by α(1,3)galactosyltransferase (1,3-Gal). This motif is located on endothelial cells of pig organs, and binding of preformed antibodies in primates leads to induction of the classical pathway of immune recognition and activation of the recipient's complement system. The role of antigal antibodies in humans and primates is unknown but may be linked to protection from intestinal flora or may facilitate clearance of aged red blood cells, which express limited amounts of similar epitopes over the course of their lifetime [4].

A major milestone in organ xenotransplantation was the development of 1,3-Gal knockout pigs [5,6]. Knockout pigs are phenotypically normal [6]. Hearts and kidneys from 1,3-Gal knockout pigs have been transplanted into nonhuman primates and recipient animals have survived for 2–6 months. Xenotransplantation of livers into nonhuman primates have been limited to less than 8 days. Even though graft survival is short, a future clinical application would be the use of a xenogeneic organ as a bridge to definitive allogeneic transplantation for patients with immediate life-threatening liver failure. Some of the most interesting results have come from the use of donor transgenic pigs with the 1,3-Gal knockout and expression of CD46 human complement regulatory protein. Recipient baboons transplanted with livers from these pigs survived for up to 7 days [7■]. Survival was limited, however, by severe thrombocytopenia that generally began within hours of liver reperfusion and led to life-threatening hemorrhage within days and persisted despite splenectomy. Liver function was preserved with evidence of porcine-derived protein expression and near normal coagulation parameters prior to dysregulation of the coagulation cascade from severe hemorrhage [8]. Current studies are directed against reversing thrombocytopenia in recipient animals. Taken together, these studies show that elimination of the galactose-α(1,3)galactose epitope is a major milestone for xenotransplantation. Now that this recognition site can be eliminated in donor pigs, its absence has also uncovered other, non-Gal epitopes that may exert an immunologic effect, albeit to a lesser extent [9].

Infectious transmission of pathogens in porcine organs or cells can be minimized by breeding pigs to be genetically homogeneous in pathogen-free herds, yet this does not eliminate the risk of transmission of porcine endogenous retroviruses (PERVs). However, over 200 patients have been transplanted or exposed to porcine-derived cells and tissues without a reported transmission of PERVs, but further controlled studies are needed to assess the risk in pig to primate xenotransplantation (reviewed in [10]).

WHOLE ORGAN LIVER TISSUE ENGINEERING

As the start of tissue engineering as a discrete discipline in the early 1990s, the liver has been the organ most frequently singled out to be "grown" in the laboratory [11,12]. Hence, it is not surprising that some of the very first experiments published in this field reported growing hepatocytes onto biodegradable sponges or scaffolds and then transplanted into rats [13].

In these initial studies, scaffolds were made of synthetic materials as many of the first researchers had experience with suture materials, plastics and textiles that demonstrated good biocompatibility and ease of use. Poly-L-lactic acid (PLA) infiltrated with polyvinyl alcohol (PVA), or PLA and poly-DL-lactic co-glycolic acid (PLGA) sponges are among the

materials initially used to construct scaffolds [13-15]. However, one of the early limitations of these experimental approaches was the lack of a vascular network to nourish and maintain the seeded hepatocytes as larger organoids were constructed. Consequentially, hepatocyte survival after implantation was significantly low with only a small fraction of cells surviving the first 7 days of implantation, the approximate time that host blood vessels grow and vascularize the implanted tissue [14].

These challenges were initially addressed by delivery of growth factors to enhance angiogenesis and increase cell viability [16,17]. Additionally, improvement in hepatocyte seeding efficiency onto scaffolds and development of advanced bioreactors with defined culture conditions, helped improve cellular function of early hepatic constructs [18].

Drug discovery and the ability to evaluate the toxicology profile of new medications in tissue engineered three-dimentional liver scaffolds were some of the applications of this new technology that gained immediate significance and popularity. With the improvement of cell culture conditions, extracellular matrix (ECM) characterization and scaffolding biomaterials, many of these constructs expressed detectable levels of cytochrome P450. The conceptual design of “liver on a chip”, or more recently “body on a chip”, is now an achievable goal [19,20]. These systems have the potential to allow for the rapid and inexpensive screening of new therapeutic drug candidates by increasing throughput efficacy and monitoring for toxicity in an all-at-once, single microsystem. Similarly, seeding of hepatocytes in micropatterned surfaces along with stromal cells enhances hepatocyte function, allowing tissue engineered constructs to retain high rates of specific hepatic metabolic function [21]. In these last decade, countless other experimental approaches and methods have also been developed using different vascularized biomaterials and cell combinations to improve engraftment, cell survival and hepatic function [22-24,25■,26,27■■].

One of the more significant limitations of initial attempts at liver tissue engineering using early synthetic scaffold design is the lack of specific hepatic cues for cell growth and differentiation and need for improved characterization of the microarchitecture.

Over the last few years, tissue and whole organ decellularization has overcome many of these limitations and early studies indicate that this may be a promising technology. Initially, the decellularization method was used for thin tissues and seeding with fresh rat hepatocytes followed [28]. These methods showed good expression of albumin and urea and demonstrated good viability at 35 days in in-vitro culture. Nevertheless, the lack of a real three-dimentional environment probably explains why the hepatocytes in these decellularized scaffolds produced less albumin and urea than the “gold standard” control of hepatocytes seeded in a collagen I gel sandwich.

More recently, Ott *et al.* [29] have developed a novel method of perfusion decellularization that provided for the foundation of whole organ scaffolds [24]. The use of this method allowed the decellularization of whole hearts, livers, pancreata, kidneys and small intestine loops leaving the natural vascular network intact. These decellularized organs could then readily be recellularized with freshly isolated cells and maintained in bioreactors for cell expansion, differentiation and function. This approach alone has the potential to considerably change the field of organ bioengineering. We and others have recently used similar perfusion decellularization techniques with the liver to generate decellularized organ scaffolds for organ bioengineering [24,25■,27■■,30]. Importantly, these bioscaffolds preserved their hepatic microarchitecture and an intact vascular system, which can be promptly used for recellularization by perfusion of culture medium with different cell populations. Uygun *et al.* [25■] decellularized rat livers and repopulated them with primary rat hepatocytes, showing promising hepatic function and ability to heterotopically transplant

these bioengineered livers into recipient rodents for up to 8 h. Baptista *et al.* [27■■] decellularized ferret livers and were able to seed these with human fetal liver progenitor and endothelial cells that were able to differentiate in a bioreactor into biliary epithelial cells that formed bile ducts and clusters of hepatocytes. These liver constructs were able to reproduce several critical functions of the liver, and acellular liver bioscaffolds could also be transplanted into rats.

Nevertheless, it is difficult to predict the future outcome and the real translational value of this technology. Particular attention needs to be given to the species of origin that provides the organs for decellularization, proper selection of cell source and the bioengineering process to grow these organs. For clinical applications, the interaction of these materials with the human host immune system remains to be evaluated. In this case, what had been assumed to be a harmless biomaterial – namely, decellularized porcine heart valves – has the potential to initiate macrophage and lymphocyte activation and immunoglobulin deposition, depending on the methods used in their preparation [31,32]. It is then imperative to validate the long-term efficacy and safety outcomes of these bioengineered organs and tissues, assuring that they do not endanger at any moment the patient's health.

LIVER CELL TRANSPLANTATION

Liver cell transplantation has been considered as a potential therapy for patients with life-threatening liver diseases, as the development of techniques to isolate individual cells from the liver [33]. The liver cell transplantation procedure simply involves the direct injection of a preparation of isolated cells into the liver via portal vein or directly into the spleen. This approach is far less invasive than orthotopic liver transplantation and can be performed safely in severely ill patients. Laboratory studies have demonstrated that isolated liver cells can engraft in the liver and other extra hepatic sites and can function to correct various metabolic deficiencies of the liver and reverse hepatic failure [34■]. Additionally, clinical trials of liver cell transplantation have demonstrated the long-term safety of the procedure [35,36]. However, only partial correction of metabolic disorders has been achieved, and the success has not yet been sufficient to elude the need for whole organ transplantation [34■].

At present, liver cell transplantation remains as an alternative, experimental treatment to bridge patients to orthotopic liver transplantation. Until now, relatively poor initial and long-term hepatocyte engraftment has limited the successful treatment of chronic diseases, such as liver-based metabolic deficiencies. Critical shortage of human donor organs, and therefore hepatocytes, is the primary limiting factor for the widespread application of this therapy.

Human hepatocytes are primary obtained from livers rejected for orthotopic liver transplantation and unused segments from volume-reduced donor livers [37]. A promising alternative source of human liver cells may come from marginal donor organs resuscitated using machine perfusion, but machine perfusion presents a variety of practical and logistical issues [38]. In this context, cryopreserved liver cells may represent a viable option. However, liver cell cryopreservation is a challenging technique [35,39,40]. Finally, xenotransplantation of liver cells could provide a limitless supply of donor organs and cells, but previous attempts in humans resulted in hyperacute rejection [41]. Surprisingly, isolated porcine hepatocytes have been shown to secrete albumin for several months in naive nonhuman primates after transplantation [34■,35,36,42].

At this point it is also not clear whether fetal hepatocytes can be induced to expand to the degree necessary for clinical application [43■] and if cells derived from fetal livers before 20 weeks of gestational age, when most elective abortions are performed, would express differentiated and sufficient hepatocellular function for transplantation [43■].

Embryonic stem cells [44] and induced pluripotent stem (iPS) [45,46] cells are pluripotent cells that can be propagated indefinitely as undifferentiated cells with a normal karyotype and can differentiate into practically any cell type including liver [47,48■]. One limitation is that the quantity of cells needed for the treatment of liver failure would require the generation of billions of hepatocytes from autologous cells derived from iPS technology that would necessitate a substantial period to evaluate purity, functional capacity and quality while excluding any tumorigenic potential. However, the use of iPS cells in studying liver development, biology and drug discovery makes them an outstanding cell source.

Conceptually, liver cell transplantation could provide rapid support for the failing liver by providing metabolism of liver toxins, secretion of proteins such as clotting factors and albumin to stabilize hemodynamic parameters. Nonetheless, results from human clinical trials have been confusing, perhaps due to the wide range in quantity and phenotype of cells transplanted, anatomical sites wherein cells have been infused (liver vs. spleen), and high spontaneous recovery rates in patients with acute liver failure [34■,35,36,49,50]. The fortunate news is that the delivery of hepatocytes appears to not have led to serious adverse events that could harm further translational efforts. These early studies have illustrated the need for clinically relevant animal models of acute liver failure and controlled trials with multiinstitutional, standardized treatment protocols.

The treatment of chronic end-stage liver disease is even more problematic. The functional and anatomical abnormalities in the cirrhotic liver make it difficult to believe that transplantation of hepatocytes into the failing liver via portal vein infusion could improve the functional state of the liver [34■,49]. Early attempts in humans with end-stage cirrhosis have provided more questions than evidence of clear improvement in hepatic function. Although, experimental models have clearly demonstrated that delivery of hepatocytes into the spleen dramatically influences engraftment and function [34■,49,51], clinical trials have failed to demonstrate its effectiveness.

Inborn errors of metabolism are the first target of liver cell therapy due to the less variable clinical conditions and objective measurements to evaluate proper hepatocyte cell mass. Liver cell transplants and laboratory data can be determined to unequivocally assess the efficacy of liver cell transplantation [34■,49,52,53]. On the contrary, metabolic liver abnormalities are rarely immediately life threatening and often-acceptable conventional medical therapies are available before a transplant is required. Therefore, the potential benefit must be carefully weighed against any possible complications [35,49]. With respect to long-term engraftment it will be important to ascertain whether the transplanted hepatocytes will gain a selection advantage over the recipient’s native cells. In the recent years, enormous effort has focused on the development of techniques to improve cell engraftment and provide growth advantages to donor cells [36]. Promising results have been generated by a number of alternatives (e.g. liver directed radiation, portal vein embolization, partial hepatic resection) with potential clinical translation [53]. Current techniques undergoing investigation as bioartificial substitutes for liver replacement are as follows:

1. extra-corporeal bioactive liver perfusion systems.
2. whole organ xenotransplantation.
3. liver tissue engineering.
4. cell transplantation.

CONCLUSION

Lately, interest in xenotransplantation and tissue engineering using decellularization techniques has increased significantly and both potentially share a common need to further clarify immune recognition of the resulting transplanted organ. New genetic knockout pigs have minimized hyperacute rejection and in the latter case, the concept of engineering liver grafts can potentially use allogeneic or xenogeneic scaffolds. Removal of antigenic cells from a discarded organ leaves open the possibility of reconstituting the scaffold with the recipient patient's own cells using cellular engineering technology. Taken together, the native liver matrix may represent the ideal structure-scaffold for cell transplantation by enhancing engraftment and differentiation. Cell transplantation could greatly benefit from organ and cell engineering techniques. The bioengineering of a transplantable scaffold that contains the necessary microstructure and extracellular cues for tissue assembly, function and differentiation is an exciting possibility that could ultimately allow the development of organs on demand for transplantation and would open a new era in the field of organ transplantation.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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KEY POINTS

- A major milestone in organ xenotransplantation was the development of 1,3-Gal knockout pigs.
- Perfusion decellularization creates acellular scaffolds from whole organs and retains the natural vascular network.
- Liver cell transplantation is an alternative, experimental treatment to bridge patients to orthotopic liver transplantation and may be particularly useful in patients with metabolic abnormalities of the liver.