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Review

Engineering Liver Therapies for the Future

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ABSTRACT

Treatment of liver disease has been greatly improved by the advent and evolution of liver transplantation. However, as demand for donor organs continues to increase beyond their availability, the need for alternative liver therapies is clear. Several approaches including extracorporeal devices, cell transplantation, and tissue-engineered constructs have been proposed as potential adjuncts or even replacements for transplantation. Simultaneously, experience from the liver biology community have provided valuable insight into tissue morphogenesis and *in vitro* stabilization of the hepatocyte phenotype. The next generation of cellular therapies must therefore consider incorporating cell sources and cellular microenvironments that provide both a large population of cells and strategies to maintain liver-specific functions over extended time frames. As cell-based therapies evolve, their success will require contribution from many diverse disciplines including regenerative medicine, developmental biology, and transplant medicine.

INTRODUCTION

DRAMATIC ADVANCES in surgical techniques and immunosuppression have permitted the use of liver transplantation in the management of liver disease. Despite resourceful use of donor livers through split liver transplantation and living related donors, patients' needs are not being met. Advances are also being made to try and provide genetically engineered xenografts for transplantation; however, this approach is still experimental. Because of the persistent donor shortage, several cell-based therapies for liver disease have been proposed; namely, extracorporeal bioartificial liver devices, cell transplantation, and tissue-engineered constructs. Despite their differences, these therapies share a requirement for adequate cell supply and stability of liver-specific functions. Below, we review both surgical organ-based approaches as well as the current state-of-the-art in cell-based approaches in this context. Although some extracorporeal cell-based support devices are already in clinical trials, the efficacy of engineered cell therapies in the management of liver disease can be improved further by incorporating strategies for maximizing hepatocyte function. To apply such strategies, lessons must be drawn from classic liver biology as well as the experience of transplant medicine in supporting liver failure patients.

SURGICAL APPROACHES

In the 1960s, individuals with liver disease had a poor prognosis and few options for effective treatment. Today, even with a better understanding of the etiology of liver disease, 1 in every 10 individuals in the United States is or has been diagnosed with liver or biliary disease. In particular, hepatitis C virus is a widespread public health problem. Worldwide, approximately 170 million people are infected with the hepatitis C virus (HCV), and each

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year 8000 to 10,000 deaths result from HCV complications in the United States alone.[1] Despite medical advances, few liver diseases are curable and the standard treatment for liver decompensation is whole organ orthotopic transplantation. Below, we survey a range of strategies for liver transplantation using cadaveric donors, split liver techniques, living related donors, and xenogeneic sources.

Cadaveric transplantation

Starzl and colleagues performed the first human liver transplantations in 1963.[2] Until the application of cyclosporine for immunosuppression in the late 1970s, the long-term survival of transplant recipients was low. Because of continued improvements in surgical techniques, organ preservation, and immunosuppression, 1-year survival rates are currently 85–90%. The most common indications for liver transplantation are chronic hepatitis, alcoholic liver disease, and cirrhosis. The standard immunosuppressive therapy for transplant recipients consists of tacrolimus plus corticosteroids. In 1999, of the 14,707 individuals on the waiting list, 4498 received transplants and 1709 died while waiting.[3] As of February 2002, 18,434 people are waiting for a liver transplantation. The increasing disparity between the number of patients awaiting transplantation and the number of available organs is a key factor motivating the development of novel therapies for liver disease.

Split liver transplantation

In efforts to more effectively distribute donated organs, surgeons have developed techniques to use a single liver in two recipients. Split liver procedures originated with reduced-size transplantation, in which a child would receive only the right lobe of a cadaveric liver. It soon became evident that the larger left lobe could be successfully grafted into adults, rather than discarded. Reports indicate that the success of split liver transplantation is close to that of whole organ transplantation.[4] The general acceptance of split liver transplantation represents an important advancement in the management and distribution of limited cadaveric organs. Nevertheless, split liver operations are not performed everywhere and may be complicated by anatomical variation or the availability of recipients meeting specified criteria.

Living donor transplantation

As with cadaveric split liver transplantation, living donor liver transplants were first performed in children in 1988.[5] Typically, children requiring a transplant may receive the left lobe from an adult, with little risk to the donor.[6] A few years after this success, adult-to-adult living donor transplants were successfully performed, using the right lobe.[7] However, right hepatic lobectomy is surgically complex and presents a greater risk to the donor.[8] Although split liver procedures with both cadavers and living donors expand the options available to physicians and patients, liver transplantation is unlikely to meet the demand for treatment and alternative therapies must be explored.

Xenotransplantation

The earliest reports of hepatic xenografts came in 1993, with baboon liver transplantation being utilized for support of fulminant hepatic failure.[9] In more recent years, the pig has been identified as an optimal organ source because of comparable liver size and unlimited source. The major problems with xenotransplantation are immune rejection and risk of xenozoonosis by infectious agents such as porcine endogenous retrovirus (PERV). Although many propose that infectious risk is minimal, *in vitro* studies have shown that PERV can infect human cells.[10] After initially halting clinical trials with pig tissues in October 1997, the Food and Drug Administration (FDA) has since permitted several trials to resume. Nonetheless, xenotransplantation guidelines published by the FDA emphasize the infectious risk of xenotransplantation.[11]

Early immune rejection of xenografts is due to the so-called hyperacute rejection response. Preexisting antibodies to carbohydrate epitopes on pig endothelium exist at high titers in humans, likely because of antigen stimulation by natural flora. Thus, complement-mediated damage of xenografts rapidly causes deendothelialization, blood vessel occlusion, and graft failure. To overcome this limitation, some investigators are breeding transgenic pigs with a modified endothelial surface. Proposed approaches modify the complement response by expression of decay-accelerating factor (DAF)[12] or human complement-inhibitory protein[13,14] or alter the expression of galactose [Gal(α1–3)Gal] on the cell surface.[15,16] Despite progress toward mitigating the hyperacute rejection response, the liver poses a unique challenge for xenograft organs: the production of a large amount of xenogeneic protein as part of normal hepatocyte function. Strategies for addressing the subsequent acute and chronic phases of immune rejection would incorporate traditional immunosuppressants coupled with attempts to induce "tolerance."

While liver transplantation continues to evolve as an effective treatment for liver disease, there is clearly a role, even a need, for the development of novel therapies. Many of these approaches are in experimental stages and can take valuable lessons from the field of transplantation medicine.

CELL-BASED APPROACHES

In response to the increasing incidence of liver disease and the relative shortage of donor organs, many investi-

gators have developed cellular therapies using isolated hepatocytes. Such approaches must consider both the source of hepatocytes and crucial stabilization of liver-specific function. Cell-based therapies that are reviewed can be generally categorized as extracorporeal devices, cell transplantation, and tissue-engineered constructs.

Cell sourcing

The choice of cell type in any cellular therapy is of paramount importance. Unfortunately, the full complement of cellular functions required to replace the liver and positively affect clinical outcomes has not been determined. For example, the mediators of hepatic encephalopathy resulting from liver decompensation are not fully understood although many theories, such as accumulation of ammonia,[17] benzodiazapine,[18] or gut-derived neurotransmitters,[19] have been proposed. Hence, functionality of cellular devices is determined by "surrogate" markers of each class of liver-specific functions including synthetic functions, metabolic functions, detoxification (phase I and II pathways), and biliary excretion. The implicit assumption is that hepatocytes capable of a wide array of known functions will also express those unmeasured (or unknown) functions that are central to their metabolic role. Tissue-engineering applications may now consider sources other than primary cells as new cell lines are developed and stem cell lineages are elucidated. Table 1 outlines some of the important issues concerning the use of various cell sources.

Primary hepatocytes are the most common cellular component in current engineered therapies. Most devices undergoing clinical evaluation use porcine hepatocytes, which are readily available but, compared with rodent models, are poorly characterized *in vitro*.[20] Whereas some functions such as albumin secretion may be stable, others such as cytochrome *P*-450 decline under standard culture conditions.[21,22] In general, primary hepatocytes require specific microenvironmental cues to maintain the hepatic phenotype *in vitro*, and, as discussed in the following section, continuing investigation of culture conditions is likely to improve the stability of primary porcine hepatocytes *in vitro* as has been the case for rodent hepatocytes.

Primary human cells are a preferred source for cellular therapies, but like whole organs, they are in limited supply. Thus far, they have been used in an extracorporeal device[23] as well as for hepatocyte transplantation.[24,25] Case reports of growth potential of hepatocytes from pediatric patients exist and advancement in techniques to cryopreserve human hepatocytes will extend their utility.[26,27] Further *in vitro* characterization of human hepatocytes will provide key information affecting the development of improved cell-based therapies.

The development of highly functional hepatocyte cell lines for use in cellular therapies is an obvious strategy to overcome the growth limitations of primary cells. A common approach to immortalizing hepatocytes is retroviral transduction of the simian virus 40 tumor antigen gene (SV40 Tag) whose gene product binds to cell cycle regulator proteins Rb and p53.[28–30] Cell lines have also resulted from spontaneous immortalization of hepatocytes in collagen gel sandwich cultures or cocultures.[31] A third type of hepatic cell line is derived from liver tumors, as in the case of HepG2.[29,32]

All these cell lines are growth competent but must be evaluated on the basis of liver-specific function and safety. Immortalized hepatocytes typically underperform primary cells and may not respond to important physiologic cues.[33,34] The primary safety concern with the use of cell lines is the transmission of oncogenic factors to the host, especially with implanted cells. Efforts to improve the safety of immortalized cells has resulted in the use of temperature-sensitive SV40 Tag,[35] Cre–*loxP*-mediated oncogene excision,[36] and integration of suicide genes such as herpes simples virus thymidine kinase (HSV-tk).[37] In the case of tumor-derived or spontaneously immortalized lines, limiting patient exposure to cells and preventing tumorgenesis may prove more difficult.

Table 1. Cell Sources for Liver Therapies

Cell source	*Critical issues*
Primary Human, xenogeneic	Sourcing, expansion, safety (PERV), phenotypic stability, immunogenicity
Immortalized SV40, telomerase, tumor-derived, spontaneously immortalized	Safety (suicide genes, tumorigenicity), efficacy, genotypic instability
Stem cells Liver progenitor, embryonic, transdifferentiation (HSC, pancreas)	Sourcing, differentiation, phenotypic instability, safety (tumorigenicity), immunogenicity

Abbreviations: HSC, Hematopoietic stem cells; PERV, porcine endogenous retrovirus; SV40, simian virus 40.

In addition to primary cells and cell lines, stem cells are being considered for use in cellular therapies for liver disease. Stem cells are self-renewing cells that have the potential to differentiate into specialized cell types. The study of liver stem cell biology is rapidly evolving; therefore, we have constructed the stem cell map in Fig. 1 to summarize the current literature. Potential stem cell sources for use in cell-based therapies are embryonic stem cells, adult liver progenitors, and transdifferentiated nonhepatic cells.[38–40] Although embryonic stem cells may ultimately provide a cell source, differentiation along the early hepatocyte lineage *in vitro* has been reported only in murine embryonic stem cells.[41] The oval cell is a "facultative," bipotential stem cell that emerges in the setting of hepatic injury coupled with the inability of the adult hepatocyte to undergo repair.[42] However, despite the fact that oval cells can be propagated *in vitro*, some transplantation studies indicate that they have less repopulation potential than mature hepatocytes.[43] "Progenitor" cells have also been isolated from adult and fetal tissues that have not been subject to an oval cell protocol.[44–47] Certain progenitor cells have been characterized as multipotent hepatic stem cells with self-renewal capability *in vitro*.[48] In addition, it appears that hematopoietic stem cells can generate hepatocytes directly as well as through an oval cell intermediate, depending on the mode of injury and the model system. This has been shown in rodent models and confirmed in humans by a retrospective study of recipients of bone marrow and liver transplantation.[49–52] Although it is not clear which stem cell source would be optimal, stem cells that can proliferate yet retain the ability to differentiate into hepatocytes would provide an ideal source for engineered cellular therapies.

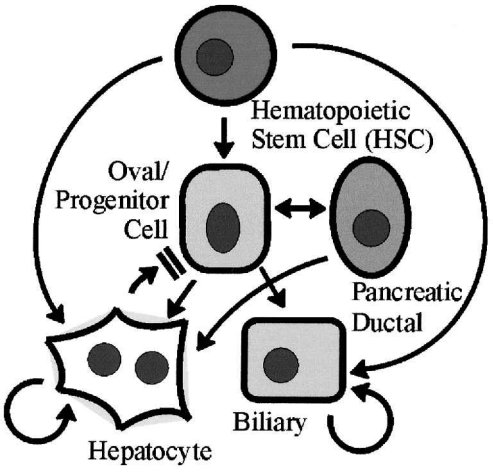

FIG. 1. Putative stem cell map of the liver. Normally, hepatocytes and biliary cells have the ability to replicate. Oval or progenitor cells are thought to be facultative stem cells. Pancreatic ductal cells can transdifferentiate into oval cells and hepatocytes. Hematopoietic stem cells may differentiate directly into hepatocytes and cholangiocytes or into oval cells, depending on the animal model.

Each of the cell sources currently under evaluation, that is, primary cells, cell lines, and stem cells, has inherent advantages and limitations. Independent of the source, mature hepatocytes in cellular therapies will likely require long-term functional stability to prove effective.

Phenotypic stability

The success of cellular therapies ultimately depends on the stability of the hepatocyte phenotype and its regulation by microenvironmental cues. Primary hepatocytes are anchorage dependent and notoriously difficult to maintain *in vitro*. Freshly isolated cells rapidly lose adult liver morphology and differentiated functions when cultured in monolayers or suspension. For years, investigators have developed culture models based on features of liver architecture to recapitulate the complex hepatocyte microenvironment seen in Fig. 2. These features include extracellular matrix as found in the space of Disse, physicochemical stimuli imposed by sinusoidal blood flow, and cell–cell interactions present in the hepatic cord. Extracellular matrix (ECM) modulation has included both variations in composition and topology.[53–58] Matrigel, a tumor-derived basement membrane-like gel, is an example of a scaffold containing varied ECM composition that has been used for hepatocyte stabilization. However, hepatocytes undergo variable aggregation on Matrigel, making it difficult to incorporate in a cell-based therapy. Sandwich culture mimicks the environment seen by hepatocytes *in vivo* by entrapping cells in between two layers of collagen gel.[59] However, such methods introduce additional transport barriers and are difficult to scale up to therapeutic levels.[60,61]

Modifications of culture media, including the addition of low concentrations of hormones, corticosteroids, cytokines, vitamins, or amino acids, are known to help stabilize hepatocyte morphology, survival, and liver-specific functions. Specifically, serum-free formulations containing epidermal growth factor (EGF), hepatic growth factor (HGF), and nicotinamide have been shown to maintain hepatocyte function and even induce proliferation *in vitro*.[47,62–64] In addition, gradients of hormones and oxygen are important modulators of hepatocyte function *in vivo* and may prove useful in designing cellular therapies.[65,66]

Finally, cell–cell interactions, both homotypic (hepatocyte–hepatocyte) and heterotypic (hepatocyte–nonparenchymal cell), have been shown to improve viability and function. Restoration of hepatocyte interactions as in spheroidal aggregates promotes formation of bile canaliculi, gap junctions, tight junctions, and E-cadherins

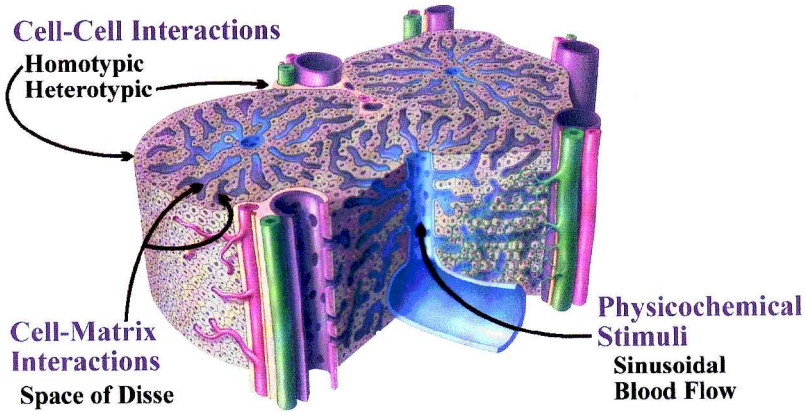

FIG. 2. Microarchitecture of liver lobule. The hepatocyte microenvironment is precisely defined with regard to cell–cell interactions, cell–matrix interactions, and biochemical stimuli (e.g., hormones). Each of these features has been utilized *in vitro* to stabilize the isolated primary hepatocyte phenotype. (Reproduced with permission from J. Daugherty.)

and stabilizes function.[67–69] The heterotypic interactions in hepatocyte–nonparenchymal cocultures are thought to present a highly conserved signal that greatly augments liver-specific functions.[70–72] Cell patterning methods have been used to study the "co-culture" effect by tightly controlling the amount of cell–cell interaction to identify specific signaling pathways.[73] Whatever the nature of the hepatocyte therapy, the issue of phenotypic stability must be addressed. Elucidation of specific molecular mechanisms that stabilize hepatocyte function would have broad impact in this field.

Extracorporeal devices

Extracorporeal support for patients suffering from liver failure has been attempted since before the 1960s (for review, see Allen *et al.*[61] and Strain and Neuberger[74]). Various nonbiological approaches such as hemodialysis or hemoperfusion over charcoal have met with limited success, presumably because the synthetic and metabolic functions of the liver are inadequately replaced in these systems.[75] Conversely, biological approaches such as hollow fiber devices, flat plate systems, perfusion beds, and suspension reactors (Fig. 3) have shown encouraging results but have been difficult to implement in the clinical setting.

The most common bioartificial liver device design incorporates hepatocytes in hollow fiber cartridges borrowed from hemodialysis. In attempts to improve the hepatocyte microenvironment, investigators have used microcarriers[76]; gel entrapment, both intraluminally[77] and in the extracapillary space[57]; multicompartment interwoven fibers[78]; and multicoaxial configurations.[79] Hollow fiber membranes provide a scaffold for cell attachment and immunoisolation, and are well characterized in a clinical setting, but may not provide adequate nutrient transport or the proper environmental cues for long-term hepatocyte stabilization. Flat plate or monolayer bioreactors have been proposed that offer better control of hepatocyte microenvironment, but would be difficult to scale up.[60,80] Many designs use perfused beds or scaffolds to promote three-dimensional architecture and minimize transport barriers. However, it may be difficult to provide uniform perfusion of the packing matrix and cells can be exposed to damaging shear forces.[81–83] Finally, encapsulated suspended cells or spheroid aggre-

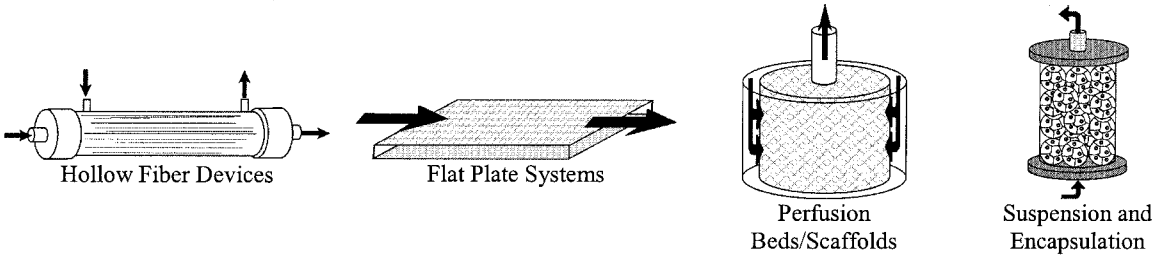

FIG. 3. Schematics of proposed extracorporeal liver support devices. Many designs have been proposed, but most fall under these four general classifications. Hollow fiber devices are the most common, but innovations in engineering, biomaterials, and fabrication have contributed to many novel perfusion- and scaffold-based systems.

gates have been incorporated in perfusion systems that would be simple to scale up, but are limited in their ability to stabilize cells.[84–86]

A successful extracorporeal bioartificial liver design will include effective bidirectional mass transport, a stable cellular microenvironment, and simple scale-up. Although many devices include a combination of convective and diffusion transport phenomena, mass transfer limitations of key nutrients to and from the cellular compartment are primarily due to diffusion resistance. Barriers to diffusive transport include membranes, collagen gels, and nonviable cells. Membranes have been used with a wide range of molecular mass cutoffs, from 20 to 200 kDa, but presently most designs specify a range between 100 and 150 kDa. As discussed in the previous section, hepatocytes require a specific microenvironment to maintain liver-specific function, something absent from many device designs. Much can be drawn from culture models that stabilize hepatocytes with cell–cell interactions, cell–matrix interactions, and chemical cues. Finally, to be clinically useful, extracorporeal devices must scale to provide efficacy. Some challenges involved in scaling up experimental reactors include maintaining a uniform microenvironment, minimizing dead volume, providing adequate cell number, and ensuring homogeneous flow distribution. In addition, establishing standard protocols for evaluating benefit to the patient and prolonging cell survival will lead to improvements of existing devices, and of the implantable approaches that will soon follow.

Cell transplantation

Cell transplantation is typically performed by intravenous or peritoneal administration of hepatocytes in suspension. This mode of cellular therapy exploits a key advantage of the *in vivo* hepatic microenvironment: adult hepatocytes engrafted in the liver or spleen can proliferate extensively and reconstitute liver function. Indeed, in a mouse model of cell transplantation, a single hepatocyte was calculated to have the potential to go through 34 population doublings, or give rise to 1.7×10^{10} cells.[87,88]

A critical element for effective regeneration is a "hepatotrophic" environment as well as available sites for cell growth. In animals, this stimulation is typically generated by partial hepatectomy, portocaval shunting, transgenic injury, or administration of hepatotoxins before cell transplantation. Delivery of the isolated cells has been attempted by injection into peripheral veins, the portal vein, or the splenic artery as well as by intraperitoneal and intrasplenic injection. The inefficient engraftment of hepatocytes ($\sim$10%) and limited cell survival remain major limitations of this technique and strategies to improve adhesion and translocation of hepatocytes into the existing liver parenchyma will undoubtedly play an important role in the future.[89] Early studies demonstrated problems with linking animal survival to function of transplanted cells—either due to unexpectedly high animal survival for relatively few cells, or beneficial effects from nonviable hepatocytes or nonhepatocytes.[75,90] Transplanted hepatocytes also require time to engraft and grow (doubling time in mice, 28 h)[91] and this may limit the utility of the procedure for certain clinical indications. An inadequate hepatocyte supply is another barrier to widespread use of this procedure. For example, metabolic defects have been replaced with relatively little liver mass (2–5%) as compared with the requirements for support of the acutely failing liver (10–30%). The normal adult liver contains approximately 10^{11} hepatocytes; therefore, even 2% of hepatocytes would require 2 billion engrafted cells.

Currently, a limited supply of allogeneic human hepatocytes is obtained by collagenase perfusion of organs deemed inappropriate for transplantation[92]; however, a number of other sources under development were discussed earlier. Studies have highlighted the possibility that the limited life span of adult hepatocytes may contribute to the development of cirrhosis.[51] Therefore, telomerase expression in differentiated human hepatocytes may extend the life span of transplanted cells *in vivo*. Cell transplantation clearly holds promise as a therapy for a subset of clinical hepatic syndromes, but faces the same challenges of cell sourcing, adequate function, and safety as other *in vivo* approaches, such as tissue-engineered constructs.

Tissue-engineered constructs

Tissue engineering of implantable cellular constructs is another emerging cellular therapy for liver disease. This approach remains largely experimental and must overcome a number of significant hurdles before it will become a viable clinical modality. The premise of this approach is similar to cell transplantation in that hepatocytes are transplanted to perform liver functions; however, hepatocytes, known to be anchorage dependent, are immobilized on scaffolds, encapsulated in aggregates, or cultured *ex vivo* to form liver "organoids" and surgically transplanted. Hepatocytes have been implanted in many sites including the peritoneal cavity and mesentery, as well as the spleen, liver, pancreas, and subcutaneous tissues.[93,94] Proposed constructs have utilized scaffolds of various chemical composition of both synthetic and biologic composition. Synthetic scaffolds include biodegradable polyesters and polysaccharides.[95–102] Biologic scaffolds have included hyaluronic acid, collagen, and more complex biomatrix.[103–107] Both scaffold architecture and chemistry clearly play a role in hepatocyte survival, morphogenesis, and function.[70,108,109] Many putatively three-dimensional scaffold architectures (e.g., microcarriers)

are effectively two-dimensional, flat surfaces from the hepatocyte perspective; therefore, functionality of implantable cellular constructs may be improved by incorporating cell culture strategies that promote three-dimensional conformations and maintain hepatocyte polarity.

Alternatively, hepatocytes have been encapsulated to promote cell aggregation and liver-specific function as well as provide immunoisolation. Encapsulation schemes have included alginate, alginate–polylysine composites,[110–113] and fibers.[114] Spheroidal hepatocyte aggregates, heterospheroids of hepatocytes and nonparenchymal cells, and cocultures formed on *in vitro* templates have been proposed as tissue organoids for implantation.[67–70,115–118] Encapsulation strategies for many different cell types, including highly metabolic hepatocytes ($V_{max} \approx 0.4$ nmol of O_2 per second per 10^6 rat cells), face a classic dilemma between restricting transport of immunomodulators while maximizing transport of nutrients and desired cell products.

Despite advances in key aspects of hepatocyte culture and understanding morphogenesis *in vitro*, tissue engineering of the liver faces significant challenges in the future.[119] It shares many of the limitations of cell transplantation (cell sourcing, immune rejection, and long-term viability) with additional issues introduced by transport limitations due to lack of hepatic vasculature, the instability of the hepatocyte phenotype when isolated from the hepatic microenvironment, and the ability for tissue structures to reorganize over time. Accordingly, fundamental research in tissue engineering has been in the metabolic requirements of hepatocytes during seeding and in early stages of implantation,[101,120–122] design of biomaterials to improve angiogenesis,[100,123,124] effects of hepatocyte microenvironment on phenotypic stability (e.g., soluble signals, cell–substrate interactions, and cell–cell interactions), and morphogenesis of hepatocyte structures in pure cultures and cocultures with nonparenchymal cells.[70,73,108] Finally, none of the current proposed constructs incorporate in their designs excretory function corresponding to the biliary system, although studies indicate that biliary morphogenesis can be achieved *in vitro*.[125]

In the future, advances in developmental biology will likely complement "brute force" strategies to replicate the exquisite microarchitecture of the liver. For example, soluble (e.g., fibroblast growth factor) and unidentified insoluble factors have been identified in differentiation of the endoderm along the hepatic lineage[126–128] as well as in branching morphogenesis of the primitive kidney.[129]

Table 2. Clinical Status of Extracorporeal Support Devices

Company	*No. of patients*	*Device*	*Phase*	*Comments*
Vitagen, La Jolla, CA (ELAD)	25	Sussman[a]	I/II; multicenter	C3A cell line, continuous treatment up to 10 days, ultrafiltrate perfusion, 150–300 mL/min, heparin, 4 replaceable cartridges, cell mass: 4×200 g[b,c]
Circe Biomedical, Lexington, MA (HepatAssist)	171	Demetriou[d]	II/III; multicenter	Cryopreserved porcine, treatment 3–6 h for 1–5 days, 400 mL/min, citrate, charcoal column, centrifugal plasmapheresis, cell mass: 50 g[e–g]
Excorp Medical, Oakdale, MN (BLSS)	5	Patzer[h]	I; one center	Primary porcine, treatment 6–30 h, whole blood perfusion, heparin anticoagulation, cell mass: 100 g
Charite Virchow Clinic–Berlin (MELS)	8	Gerlach[i]	I/II; multicenter	Primary porcine, continuous treatment up to 3 days, filtration plasmapheresis, 100 mL/min, heparin anticoagulation, cell mass: 500 g

Abbreviations: BLSS, Bioartificial liver support system; ELAD, extracorporeal liver assist device; MELS, modular extracorporeal liver system.

[a]See Sussman *et al.*[132]
[b]See Millis *et al.*[133]
[c]See Ellis *et al.*[134]
[d]See Rozga *et al.*[76]
[e]See Demetriou.[20]
[f]See Stevens *et al.*[135]
[g]See Mullon and Pitkin.[136]
[h]See Mazariegos *et al.*[137]
[i]See Gerlach *et al.*[78]

As subsequent signaling molecules are identified, these may be incorporated into tissue-engineering strategies to harness the hepatocyte as an active component of cell-based therapies.

CURRENT CLINICAL STATUS OF ENGINEERED THERAPIES

Extracorporeal devices are first on the track to clinical application, although their efficacy has yet to be fully determined. Experimental devices using suspended primary hepatocytes were among the first to be used with human patients in the late 1980s, but have met with limited success.[130,131] Presently, several hollow fiber devices are under evaluation in clinical trials (Table 2[20,76,78,132–137]). The most extensively tested device, the HepatAssist System from Circe Biomedical (Lexington, MA), completed phase II/III trials with patients. Preliminary results show improvement in 30-day survival to 71% for treated groups, compared with 62% for those receiving standard care ($n = 171$).[135] Although an examination of study subpopulations and secondary end points shows moderate benefit of the device, a conclusive measure of efficacy is confounded by factors such as transplantation, disease etiology, and stage of encephalopathy. Critical evaluation of the complete results of the HepatAssist trial should provide valuable insight for future large-scale clinical studies. Careful consideration needs to be given to treatment indications, clinical end points, and device regulation in clinical trial design so that clear evidence of treatment efficacy may be established. Ongoing clinical experiences with extracorporeal support will likely play a key role in the improvement of next-generation devices.

Cell transplantation and implantable constructs have thus far seen limited use clinically. Although cell transplantation studies are ongoing in many animal models,[40,94] only a few investigators have used them in humans to compensate for acute liver failure.[24,25] To date, there has been no report of the use of a tissue-engineered construct to treat liver disease in humans. As discussed, hepatocyte transplantation and tissue constructs face issues of optimizing transplantation site, nutrient supply, cell viability, and grafting efficiency before clinical safety and efficacy can be evaluated.

CONCLUSION

Since the 1980s, the standard treatment for liver failure has been whole organ transplantation. Improvements in surgery have allowed split liver and liver-related donor procedures to partially alleviate the shortage in organ supply. However, cell-based therapies hold promise to provide an important adjunctive treatment (i.e., bridge to liver transplantation) or eventual curative therapy in cases of metabolic defects.

Current cell-based approaches will rely on a variety of cell sources, whether primary or stem cells, which will ultimately interact with the microenvironment en route to providing key liver-specific functions. A fundamental understanding of the cues that promote phenotypic stability and tissue morphogenesis will undoubtedly contribute to the next generation of extracorporeal devices, cell transplantation therapies, and tissue-engineered constructs. Furthermore, strategies to harness and regulate host liver regeneration could even offer the potential to reverse chronic liver fibrosis and cirrhosis, currently thought to be irreversible.

In addition, immunological issues will be an important consideration for cell-based therapies; therefore, contributions from transplantation immunology that aim to promote graft tolerance are of great interest.[138] Finally, development of predictive animal models to evaluate liver therapies will offer vital preclinical assessment of new therapies as they emerge.

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