CHAPTER 46

Hepatic Tissue Engineering

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LIVER FAILURE AND CURRENT TREATMENTS

Liver disease afflicts over 600 million people worldwide, 30 million of whom are Americans. Liver disease leads to the death of over 40,000 individuals in the United States every year. Liver failure can be generally separated into two major categories: fulminant hepatic failure, also referred to as acute liver failure, and chronic hepatic failure resulting from chronic end-stage liver disorders. The term fulminant hepatic failure is utilized for cases in which hepatic encephalopathy and impaired synthetic function (i.e., coagulopathy) develops within 26 weeks of the initial onset of jaundice. Hepatic encephalopathy is a neuropsychiatric condition, which can be divided into four stages ranging from minor effects such as mild confusion and sleep disorder, to deep coma. Although fulminant hepatic failure is relatively rare, with approximately 2,000 cases in the United States per year, it exhibits a high mortality rate of approximately 28% [1]. The major identified causes of fulminant hepatic failure include acetaminophen overdose, idiosyncratic drug reactions, and viral hepatitis A and B [1]. Of note, a recent multicenter etiology study showed that 17% of fulminant hepatic failure cases remained of indeterminate origin [2]. In addition to hepatic encephalopathy, other clinical manifestations of fulminant hepatic failure include bacterial and fungal infection, coagulopathy, as well as metabolic, cardiorespiratory, and hemodynamic abnormalities. Though spontaneous recovery has been observed due to the regenerative capacity of the liver, this type of recovery is difficult to predict, and rarely occurs in various etiologies, such as idiosyncratic drug toxicity and hepatitis B [1]. Liver transplantation is currently the only therapy shown to directly alter mortality, and therefore, is the standard of care in most clinical settings. As a result, all fulminant hepatic failure patients that meet the criteria for orthotopic liver transplant are immediately listed as United Network for Organ Sharing Status 1 (highest priority) upon presentation. Factors that preclude this designation are irreversible brain damage, unresponsive cerebral edema, uncontrollable sepsis, malignancy, and multisystem organ failure. Despite the effectiveness of liver transplant in improving short-term survival of fulminant hepatic failure patients, the utility of this approach remains limited due to the scarcity of donor organs.

Liver failure due to chronic liver diseases, while exhibiting a longer time-course of disease pathogenesis, is much more common than fulminant failure, with chronic liver disease
and cirrhosis being the twelfth leading cause of death (1.3% of total deaths) in the United States in 2010 [3]. The most common causes of chronic liver disease are hepatitis B virus, hepatitis C virus, and alcohol-induced and non-alcoholic fatty liver disease (NAFLD) [4]. Other etiologies largely include metabolic and autoimmune etiologies: primary sclerosing cholangitis, primary biliary cirrhosis, α1-antitrypsin deficiency, autoimmune hepatitis, hereditary hemochromatosis, Wilson’s disease, and liver cancer. The prevalence of hepatitis C infection in the United States population has been estimated at 1.8%, or nearly 4 million individuals [5]. Notably, cirrhosis initiated by hepatitis C infection is the most frequent cause for liver transplantation, accounting for 40–50% of both individuals who have undergone transplant and those on the waiting list [6]. In addition, the long-term inflammation precipitated by chronic hepatitis B and C infection can also promote the development of hepatocellular carcinoma. As a result, substantial efforts are focused on understanding hepatitis B and C viral pathogenesis and the development of approaches for the improved control of the viruses before and after transplantation. Taken together, so-called fatty liver diseases also comprise a major proportion of chronic liver disease patients [4]. In particular, NAFLD is an increasingly prevalent condition in the United States; present in approximately 20% of adults, of which a subset (2–3% of adults) exhibit non-alcoholic steatohepatitis (NASH), defined by the presence of characteristic injury and necroinflammatory changes in addition to excessive fat accumulation. NAFLD pathogenesis has been shown to be associated with risk factors and conditions such as obesity, type 2 diabetes mellitus, hyperlipidemia, hyperinsulinemia, and insulin resistance. Collectively, chronic liver disorders can progress towards the eventual development of cirrhosis and sequelae of decompensated cirrhosis: ascites, portal hypertension, variceal bleeding, and hepatic encephalopathy. Unfortunately, these complications represent decompensation of liver function and evidence of liver dysfunction. Medical management can minimize the impact on patient quality of life but transplantation is the only currently effective therapy. As a means to more accurately determine organ allocation to patients on the liver transplant waiting list the MELD (mathematical model for end-stage liver disease) system was implemented in 2002, which assigns a priority score based on three prognostic indicators, bilirubin level, creatine level, and INR (a measure of blood clotting time) [6]. Although overall improvements in liver allocation have been achieved following the introduction of the MELD system, regional variations in MELD scores exist, and the ability of this model to accurately predict outcomes across the entire score distribution and for distinct pathologies is less clear.

Unlike other major causes of mortality, liver disease death rates are rising rather than declining. Hepatitis B and hepatitis C virus infect 370 million and 130 million people, respectively. Current hepatitis B vaccination campaigns may help to decrease hepatitis B virus (HBV) associated morbidity although the rapid increase in HIV coinfected patients has represented a challenge for clinical management. The rising rates of obesity have also resulted in the rapid rise of NAFLD worldwide with recent epidemiologic studies suggesting a prevalence rate from 6% to 35% with a median of 20%. Up to 20% of patients with NAFLD may progress to cirrhosis, raising the possibility that liver disease may become one of the most common causes of mortality worldwide. Moreover, patients with cirrhosis or hepatitis B are at greater risk of developing hepatocellular carcinoma. Consequently, hepatocellular carcinoma is the third and sixth most common cause of cancer death among men and women, respectively.

Given the steady rise in patients with liver disease, the need for liver transplantation has continued to increase. However, the number of available donor livers has not changed significantly in five years. As a result several different approaches have been undertaken to address this growing of donor organs. Several surgical options have been pursued including the use of non-heart-beating donors or split liver transplants from cadaveric or living donors [7]. Split liver transplants depend on the significant regeneration capacity of the mammalian liver. This regenerative process has been extensively examined via experiments in rodent models, which demonstrate that partial hepatectomy or chemical injury induces the
proliferation of the existing mature cell populations within the liver including hepatocytes, bile duct epithelial cells, and others, resulting in the replacement of lost liver mass. However, liver regeneration is difficult to predict clinically, and although partial liver transplants have demonstrated some effectiveness, biliary and vascular complications are major concerns in these procedures [7]. In addition, the surgical risk to the living donors (which has included several donor deaths) has raised significant ethical questions. Furthermore, in spite of these surgical advances and improvements in organ allocation, the increasing divergence between the number of patients awaiting transplantation and the number of available organs suggests that it is unlikely that liver transplantation procedures alone will expand the supply of donor livers to meet the increasing demand. Alternative approaches are therefore needed and are actively being pursued. These include several non-biological extracorporeal support systems that will be discussed in more detail later in this chapter such as plasma exchange, plasmapheresis, hemodialysis, molecular adsorbents recirculation system, or hemoperfusion over charcoal or various resins. These systems have shown limited success likely due to the narrow range of functions inherent to each of these devices relative to the complex array of functions performed by the healthy liver, which include detoxification, synthetic, and metabolic processes. Recapitulation of a substantial range of liver functions will be required to provide sufficient liver support, and it is unclear which known liver functions (e.g., gluconeogenesis, serum protein production, coagulation factor production, toxin-mediating encephalopathy) should be prioritized to improve clinical outcomes. Given this complexity, extracorporeal support systems that do not incorporate a hepatocyte component may not be able to ameliorate and augment liver disease outcomes as illustrated by the failure of several non-biological extracorporeal support systems. In contrast, limited hepatocyte transplantation trials have been shown in some cases to ameliorate and improve liver function. These cellular therapies encompass approaches at providing temporary support such as extracorporeal bioartificial liver (BAL) devices as well as more permanent adjunct interventions such as cell transplantation, transgenic xenografts, and implantable hepatocellular constructs (Fig. 46.1). Collectively, the development of these types of cell-based therapies for liver disease is a major aim of liver tissue engineering, and fundamental advances and current status of these approaches are reviewed in this chapter.

**FIGURE 46.1**

Cell-based therapies for liver disease. Extracorporeal devices perfuse patient’s blood or plasma through bioreactors containing hepatocytes. Hepatocytes are transplanted directly or implanted on scaffolds. Transgenic animals are being raised in order to reduce complement-mediated damage of the endothelium [114].
CELL SOURCES FOR LIVER CELL-BASED THERAPIES

Cell-based therapies hold great promise, but their clinical use has been hindered by the inability of hepatocytes isolated from the in vivo hepatic microenvironment to maintain hepatocyte-specific phenotype and function in vitro. Due to the paucity of human liver tissue as a cell source, alternative cell sources have been explored (Table 46.1) with inherent strengths and drawbacks. However, the criteria to characterize these alternative cell sources as a hepatocyte or hepatocyte-like cells have not been standardized and vary greatly among different studies. No single test has been demonstrated to be sufficient to determine whether a particular cell type truly recapitulates hepatocyte function; as a result, several tests must be carried out to query various domains of hepatocyte function, including bile production, detoxification, metabolic, and synthetic functions (Table 46.2).

Cell lines

Immortalized hepatocyte cell lines such as HepG2 (human hepatoblastoma) [8], the HepG2 derived line C3A [9], HepLiu (SV40 immortalized) [10], or immortalized fetal human hepatocytes [11], have been utilized as readily available surrogates for hepatic tissue. However, these cell lines lack the full functional capacity of primary adult hepatocytes, and for clinical applications there is a risk that oncogenic factors or transformed cells could be transmitted to the patient. Thus, the generation of conditionally immortalized lines and the incorporation of inducible suicide genes have been considered as potential precautionary measures.

Primary cells

The use of primary hepatocyte-based systems could potentially eliminate the issues faced by immortalized lines, and provide the appropriate repertoire of liver functions. Primary porcine hepatocytes have been utilized in a range of BAL device configurations with some encouraging results. However, the utility of xenogeneic porcine cells for human liver therapies is restricted by immunogenicity and the potential for xenozoontic transmission of infectious agents such as porcine endogenous retrovirus (PERV). Recognizing these concerns, recent efforts have led to the development of PERV-free pigs as well as genetically modified pigs that are transgenic for human proteins, thereby decreasing their immunogenicity.

Primary human hepatocytes are the ideal cell type for cell-based therapies, and the development of primary hepatocyte-based approaches is the focus of substantial ongoing research. However, progress has been hindered by the limited supply of primary human hepatocytes and the difficulty of maintaining hepatocyte function in vitro. Discussed in more detail later, hepatocytes exhibit a loss of liver-specific functions under many conditions in vitro and despite their significant proliferative capacity during regenerative responses in vivo, mature hepatocyte proliferation in culture is limited [12]. A variety of techniques have been developed

<table>
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<tr>
<th>TABLE 46.1</th>
<th>Cell sources for liver therapies</th>
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<tr>
<td><strong>Cell source</strong></td>
<td><strong>Critical issues</strong></td>
</tr>
<tr>
<td>Primary hepatocytes</td>
<td>Sourcing, expansion, phenotypic instability, immunogenicity, safety (xenozoontic)</td>
</tr>
<tr>
<td>Human adult and fetal, xenogeneic</td>
<td></td>
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<tr>
<td>Immortalized hepatocyte lines</td>
<td>Range of functions, genomic instability, safety (tumorigenicity)</td>
</tr>
<tr>
<td>Tumor-derived, SV40, telomerase, spontaneously immortalized</td>
<td></td>
</tr>
<tr>
<td>Stem Cells</td>
<td>Sourcing, differentiation efficiency, phenotypic instability, immunogenicity, safety (tumorigenicity)</td>
</tr>
<tr>
<td>Embryonic, liver progenitors (hepatoblasts, oval cells), other lineages (HSC, MAPC), induced pluripotent stem cells, direct reprogramming to hepatocytes</td>
<td></td>
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to enable the cryopreservation of human hepatocytes [13]. This enables the large number of hepatocytes that are prepared from a single liver to be stored and thawed with reproducible cellular function. This option has opened the door to a variety of in vitro pharmacologic and infectious disease studies [14–16].

Due to the limitations in mature hepatocyte expansion in vitro, alternative cell sources are being pursued. These include various stem cell and progenitor populations, which can self-renew in vitro and exhibit multipotency or pluripotency and thereby serve as a possible source of hepatocytes, as well as other non-parenchymal liver cells.

**TABLE 46.2 Hepatic functions**

<table>
<thead>
<tr>
<th>Functional classification</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Synthetic</td>
<td>Albumin Secretion, Alpha-1-antitrypsin Secretion, Coagulation Factor Production (II, IX, X), Lipoprotein and apoprotein synthesis, Ceruloplasmin production, Ferritin production, Complement production</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Ureagenesis and metabolism, Bilirubin Metabolism, Steroid Metabolism, Gluconeogenesis/Glycogen Production, Lipid metabolism</td>
</tr>
<tr>
<td>Detoxification pluripotent stem cells, direct reprogramming to hepatocytes</td>
<td>Metabolize, detoxify, and inactivate exogenous and endogenous compounds via cytochrome P450 enzymes, methyltransferases, sulfotransferases, acetyltransferases, UDP-glucuronosyltransferases, and Glutathione S-transferases</td>
</tr>
<tr>
<td>Bile Production</td>
<td></td>
</tr>
</tbody>
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Fetal and adult progenitor cells

A variety of fetal and adult progenitor cell types have been explored. Current investigations are focused on determining the differentiation potential and lineage relationships of these populations. Fetal hepatoblasts are liver precursor cells present during development that exhibit a bipotential differentiation capacity, defined by the capability to generate both hepatocytes and bile duct epithelial cells. Sourcing problems associated with fetal cells have led researchers to search for resident cells that have progenitor properties in the adult. Towards this end, a few groups have argued that rare resident cells (which may represent embryonic remnants) exhibit properties consistent with the hypothesized adult hepatic stem cells and share phenotypic markers and functional properties with fetal hepatoblasts. In adult animal livers suffering certain types of severe and chronic injury, such oval cells can mediate liver repair through a program similar to hepatic development. Whether or not these processes occur in normal human liver injury processes remains controversial. In either case, there is little evidence that cells other than adult hepatocytes participate in the daily turnover of healthy, undamaged rodent or human liver and therefore the existence and role of hepatic stem cells or progenitors in healthy rodent and human liver homeostasis remains controversial.

Along similar lines, Weiss and colleagues have demonstrated the development of bipotential mouse embryonic liver (BMEL) cell lines derived from mouse E14 embryos that exhibit characteristics comparable to fetal hepatoblasts and oval cells [17]. These BMEL cells are proliferative, can be induced to be hepatocyte-like or bile duct epithelial-like in vitro, and can...
home to the liver to undergo bipotential differentiation *in vivo* within a regenerative environment. More recently, biopotential human embryonic liver cells have been isolated and, similar to mouse BMEL cells, are proliferative and capable of bipotential differentiation \[18\].

**Pluripotent stem cells**

Human embryonic stem cells (hES) first isolated by James Thomson in 1998 offer the ability to generate large numbers of pluripotent cells that have the potential to form all three germ layers and differentiated cells including hepatocytes. Subsequently, Yamanaka and colleagues demonstrated that fully differentiated adult cells, such as fibroblasts or skin cells, could be reprogrammed to a undifferentiated, pluripotent state similar to embryonic stem cells through the forced expression of reprogramming factors Oct3/4 and Sox2 along with either Klf4 or Nanog and Lin28. These reprogrammed cells are termed induced pluripotent stem (iPS) cells and highly resemble hES cells, sharing many characteristics such as self-renewal capabilities *in vitro* and pluripotent differentiation potential *in vitro* and *in vivo* (though epigenetic differences between hES and iPS have been identified). Since iPS cells are sourced from adult somatic cells unlike embryonic stem cells, ethical considerations associated with hES are eliminated. Moreover, iPS cells permit the generation of patient-specific cell populations, potentially enabling therapies to be developed according to the characteristics of an individual patient or to study a variety of metabolic disorders and genetic variations that only manifest in an adult.

Inspired by the understanding of how a totipotent stem cell proceeds to become a hepatocyte during normal development, multiple protocols have been described that enable hES cell differentiation into hepatocyte-like cells. Duncan and colleagues, as well as other researchers, demonstrated that through iPS reprogramming and a subsequent multistep differentiation protocol, skin fibroblasts can give rise to hepatocyte-like cells, which not only exhibit a variety of hepatocyte-specific functions *in vitro*, but can also be induced to generate intact fetal livers in mice *in vivo* via tetraploid complementation \[19\]. Pluripotent stem cell-derived hepatocytes exhibit both the phenotypic and functional characteristics associated with hepatocytes, though the resulting differentiated cells exhibit a more fetal state of maturation compared to adult human hepatocytes \[20\]. In addition, as with adult primary hepatocytes, it is not clear whether stem cell-derived ‘hepatocyte-like cells’ will maintain their phenotype *in vitro*. To date, only one example of *in vivo* engraftment of pluripotent stem cell-derived hepatocyte-like cells has been reported; this group utilized a unique and specific injury mouse model, and the authors noted little to no human-specific hepatic functions after engraftment \[21\]. Future studies will need to verify that pluripotent stem cell-derived hepatocyte-like cells can engraft *in vivo*, and will also need to address safety concerns, such as the potential for pluripotent cell-derived teratoma formation, and the oncogenic risks associated with integrating vectors used to generate some iPS lines. Human embryonic stem (ES) cells have recently been approved by the FDA (Food and Drug Administration) for safety trials to commence such studies.

Over the past few years a variety of lineages have been generated via the direct reprogramming of one adult cell type into another, without an undifferentiated pluripotent intermediate. Similar to the use of master transcriptional regulators in the reprogramming to iPS cells, the expression of a key sets of genes have been used to directly generate skeletal muscle tissue, cells that highly resemble β-cells, and neurons \[22\]. More recently, mouse fibroblasts have been directly reprogrammed to generate hepatocytes \[23,24\]. These findings raise future possibilities for deriving human hepatocytes directly from another adult cell type, although it is unclear how reprogrammed hepatocyte-like cells may behave compared to primary cells, or whether this approach will also be successful using human cells.

Ultimately, understanding the mechanisms governing the fates of stem and progenitor cell populations can empower the development of cell-based therapies. However, many challenges remain, including the ability to program differentiation completely, beyond a fetal hepatocyte stage. Furthermore, regardless of the cell source, phenotypic stabilization of
hepatocytes ex vivo remains a primary issue. Microenvironmental signals including soluble mediators, cell-extracellular matrix interactions, and cell-cell interactions have been implicated in the regulation of hepatocyte function. Accordingly, the development of robust in vitro liver models is an essential stepping-stone towards a thorough understanding of hepatocyte biology and improved effectiveness of cell-based therapies for liver disease and failure.

**IN VITRO HEPATIC CULTURE MODELS**

An extensive range of liver model systems have been developed, some of which include: perfused whole organs and wedge biopsies; precision cut liver slices; isolated primary hepatocytes in suspension or cultured upon extracellular matrix; immortalized liver cell lines; isolated organelles, membranes or enzymes; and recombinant systems expressing specific drug metabolism enzymes [25]. While perfused whole organs, wedge biopsies, and liver slices maintain many aspects of the normal in vivo microenvironment and architecture, they typically suffer from short-term viability (≤ 24 hours) and limited nutrient/oxygen diffusion to inner cell layers. Purified liver fractions and single enzyme systems are routinely used in high-throughput systems to identify enzymes involved in the metabolism of new pharmaceutical compounds; although they lack the complete spectrum of gene expression and cellular machinery required for liver-specific functions. In addition, cell lines derived from hepatoblastomas or from immortalization of primary hepatocytes are finding limited use as reproducible, inexpensive models of hepatic tissue. However, such lines are plagued by abnormal levels and repertoire of hepatic functions [26], perhaps most notably, the divergence of constitutive and inducible levels of cytochrome P450 enzymes [27]. Though each of these models has found utility for focused questions in drug metabolism research, isolated primary hepatocytes are generally considered to be most physiologically relevant for constructing in vitro platforms for a multitude of applications. However, a major limitation in the use of primary hepatocytes is that they are notoriously difficult to maintain in culture due to their precipitous decline in viability and liver-specific functions upon isolation from the liver [25]. Accordingly, substantial research has been conducted over the last two decades towards elucidating the specific molecular stimuli that can maintain phenotypic functions in hepatocytes. In subsequent sections, we present examples of strategies that have been employed to improve the survival and liver-specific functions of primary hepatocytes in culture.

**In vivo microenvironment of the liver**

In order to engineer an optimal microenvironment for hepatocytes in vitro, one can utilize as a guide the precisely defined architecture of the liver, in which hepatocytes interact with diverse extracellular matrix molecules, non-parenchymal cells, and soluble factors (i.e., hormones, oxygen) (Fig. 46.2). Structurally, the two lobes of the liver contain repeating functional units called lobules, which are centered on a draining central vein. Portal triads at each corner of a lobule contain portal venules, arterioles and bile ductules. The blood supply to the liver comes from two major blood vessels on its right lobe: the hepatic artery (one-third of the blood) and the portal vein (two-thirds). The intrahepatic circulation consists of sinusoids, which are small tortuous vessels lined by a fenestrated basement membrane lacking endothelium that is separated from the hepatocyte compartment by a thin extracellular matrix region termed the space of Disse. The hepatocytes, constituting ~70% of the liver mass, are arranged in unicellular plates along the sinusoid where they experience homotypic cell interactions. Several types of junctions (i.e., gap junctions, cadherins, and tight junctions) and bile canaliculi at the interface of hepatocytes facilitate the coordinated excretion of bile to the bile duct and subsequently to the gall bladder. Non-parenchymal cells including stellate cells, cholangiocytes (biliary ductal cells), sinusoidal endothelial cells, Kupffer cells (liver macrophages), natural killer cells, and pit cells (large granular lymphocytes) interact with hepatocytes to modulate their diverse functions. In the space of Disse, hepatocytes are
sandwiched between layers of extracellular matrix (collagen types I, II, III, IV, laminin, fibronectin, heparan sulfate proteoglycans), the composition of which varies from the portal triad to the central vein [28].

Within the liver lobule, hepatocytes are partitioned into three zones based on morphological and functional variations along the length of the sinusoid (zonation). Zonal differences have been observed in virtually all hepatocyte functions. For instance, compartmentalization of gene expression is thought to underlie the capacity of the liver to operate as a ‘glucostat’. Furthermore, zonal differences in expression of cytochrome-P450 enzymes have also been implicated in the zonal hepatotoxicity observed with some xenobiotics [29]. Possible modulators of zonation include blood-borne hormones, oxygen tension, pH levels, extracellular matrix composition, and innervation [30]. Therefore, a precisely defined microarchitecture, coupled with specific cell-cell, cell-soluble factor and cell-matrix interactions allows the liver to carry out its many diverse functions, which can be broadly categorized into protein synthesis (i.e., albumin, clotting factors), cholesterol metabolism, bile production, glucose and fatty acid metabolism, and detoxification of endogenous (i.e., bilirubin, ammonia) and exogenous (drugs and environmental compounds) substances.

Two-dimensional cultures

Numerous studies have improved primary hepatocyte survival and liver-specific functions \textit{in vitro} through modifications in microenvironmental signals including soluble factors (medium composition), cell-matrix interactions as well as heterotypic cell-cell interactions with non-parenchymal cells (Fig. 46.3). The supplementation of culture media with

![Diagram of liver lobule](image)

**FIGURE 46.2**
The precisely defined architecture of the repeating unit of the liver, the lobule. Hepatocytes are arranged in cords along the length of the sinusoid where they interact with extracellular matrix molecules, non-parenchymal cells and gradients of soluble factors. Nutrient and oxygen rich blood from the intestine flows into the sinusoid via the portal vein. After being processed by the hepatocytes, the blood enters the central vein and into the systemic circulation. (Reproduced with permission from J. Daugherty.)

**FIGURE 46.3**
Cell sourcing of human hepatocytes. Approaches have focused on modulating \textit{in vitro} culture conditions, such as the addition of soluble factors such as growth factors and small molecules, extracellular matrix proteins, heterotypic cell-cell interactions.
physiological factors such as hormones, corticosteroids, growth factors, vitamins, amino acids or trace elements [25,31], or non-physiological small molecules such as phenobarbital and dimethylsulfoxide [32,33] have been shown to modulate hepatocyte function. More recently, oxygen tension has also been shown to modulate hepatocyte function [34]. Certain mitogenic factors like epidermal growth factor and hepatocyte growth factor can induce limited proliferation in rat hepatocytes in vitro, but these mitogens result in negligible proliferation of human hepatocytes in vitro [35,36]. The impact of extracellular matrix composition and topology on hepatocyte phenotype is widely recognized. A variety of extracellular matrix (ECM) coatings (most commonly collagen type I) have been shown to enhance hepatocyte attachment to the substrate, but this usually occurs concomitantly with hepatocyte spreading and a subsequent loss of hepatocyte function [37]. Studies investigating the benefits of complex mixtures of ECM components have utilized strategies including Matrigel, liver-derived biomatrix [37–40], or bottom-up approaches such as a combinatorial high-throughput ECM microarray, where the latter has revealed specific combinations of ECM molecules that improve hepatocyte function compared to monolayer cultures on collagen I [41]. In contrast to culturing hepatocytes on a monolayer of ECM molecules, hepatocytes have also been sandwiched between two layers of type I collagen gel. In this classic ‘sandwich’ culture format, hepatocytes exhibit desirable morphology with polarized bile canaliculi, as well as stable functions for several weeks [42,43]. However, phase I/II detoxification processes have been shown to become imbalanced over time in this format [44]. Also, the presence of an overlaid layer of extracellular matrix may present diffusion barriers for molecular stimuli (i.e., drug candidates), and the fragility of the gelled matrix may hinder scale-up within BAl devices or multiwell in vitro models.

Heterotypic interactions between hepatocytes and their non-parenchymal neighbors are known to be important at multiple stages in vivo. Liver specification from the endodermal foregut and mesenchymal vasculature during development is believed to be mediated by heterotypic interactions [45,46]. Similarly, non-parenchymal cells of several types modulate cell fate processes of hepatocytes under both physiologic and pathophysiologic conditions within the adult liver [47,48]. Substantial work pioneered by Guguen-Guillouzo and colleagues has demonstrated that a wide variety of non-parenchymal cells from both within and outside the liver are capable of supporting hepatocyte function for several weeks in co-culture contexts in vitro, even across species barriers, suggesting that the mechanisms responsible for non-parenchymal cell-mediated stabilization of hepatocyte phenotype may be conserved [49,50]. Microfabrication approaches have been employed to control tissue microarchitecture in hepatic co-cultures so as to achieve an optimal balance of homotypic and heterotypic cellular interactions to promote hepatocyte function, which will be described in a later section. Hepatic co-cultures have been utilized to investigate various physiologic and pathophysiologic processes, including the acute phase response, mutagenesis, xenobiotic toxicity, oxidative stress, lipid and drug metabolism [50]. For example, co-cultures of hepatocytes and Kupffer cells have been used to examine mechanisms of hepatocellular damage [51,52], while co-cultures with liver sinusoidal endothelial cells (which are also phenotypically unstable in monoculture upon isolation from the liver) have highlighted the importance of hepatocyte-endothelial cell interactions in the bidirectional stabilization of these cell types [53,56]. Studies focused on the underlying mechanisms of hepatocyte stabilization in co-culture have identified several cell surface and secreted factors that play a role including T-cadherin, E-cadherin, decorin and TGF-β1, which demonstrate the potential for highly functional hepatocyte-only culture platforms [57,58].

**Three-dimensional cultures**

Culture of hepatocytes on substrates that promote aggregation into three-dimensional spheroids has also been extensively explored. Numerous technologies including non-adhesive surfaces (such as poly(2-hydroxyethyl methacrylate) and positively charged Primaria dishes),
rotational or rocking cultures, and encapsulation in macroporous scaffolds have been developed for hepatocyte spheroid formation [59–64]. Under spheroidal culture conditions, hepatocyte survival and functions are improved over standard monolayers on collagen [65]. Potential mechanisms underlying these effects include an increased extent of homotypic cell-cell contacts between hepatocytes, the retention of a three-dimensional cytoarchitecture, and the three-dimensional presentation of ECM molecules around the spheroids [59]. Some limitations of conventional spheroidal culture include a tendency for secondary aggregation of spheroids and the resultant development of a necrotic core in the larger aggregates due to diffusion-limited transport of nutrients and waste, and the lack of control over the cell numbers within each spheroid [25]. A variety of methods are being developed to prevent secondary aggregation of initially-formed spheroids and control cell-cell interactions, including microfabricated scaffolds, bioreactor systems, encapsulation techniques and synthetic linkers [26,66,67–73], which are discussed in further detail in the following sections.

**Bioreactor cultures**

A wide range of small-scale bioreactor platforms have been developed for *in vitro* liver applications. For example, perfusion systems containing hepatocellular aggregates exhibit desirable cell morphology and liver-specific functions for several weeks in culture [26,68,74–75], and the incorporation of multiple reactors in parallel has been explored as an approach for high-throughput drug screening studies [76–79]. In order to promote oxygen delivery while protecting hepatocytes from deleterious shear effects, gas-permeable membranes with endothelial-like physical parameters, grooved substrates and microfluidic microchannel networks have been integrated into several bioreactor designs [76,80–82]. *In vivo*, hepatocyte functions are heterogeneousy segregated along the hepatic sinusoid, and this zonation is thought to be modulated by gradients in oxygen, hormones, nutrients and extracellular matrix molecules. Using a parallel-plate bioreactor, it was demonstrated that steady state oxygen gradients characteristic of those found in liver sinusoids could contribute to a heterogeneous expression of drug metabolism enzymes CYP2B and CYP3A in hepatocyte-non-parenchymal co-cultures, which mimics the expression gradients present *in vivo* (Fig. 46.4) [83]. Furthermore, the localization of acetaminophen-induced hepatic toxicity in the region experiencing low oxygen in this model system recapitulates the perivenous location of toxicity *in vivo*. A zonal microbioreactor array based on microfluidic serpentine mixing regions feeding into several microbioreactors in parallel has also been designed for potential *in vitro* liver zonation applications (Fig. 46.4). Overall, the ability to decouple oxygen tension from gradients of other soluble stimuli and cell-cell interaction effects within this platform represents an important tool for the systematic investigation of the role of extracellular stimuli in zonation.

Bioreactors may also be used to study more dynamic physiological processes than is possible in conventional culture platforms. For example, a recent bioreactor device describes the ability to monitor invasion of metastatic cells into hepatic parenchyma by recreating relevant features of the liver tissue such as fluid flow and length scales [84]. Collectively, by providing dynamic control over hepatocyte culture parameters and hence hepatocyte function, bioreactors will continue to be useful in studying liver biology and facilitating drug development applications.

**Microtechnology tools to optimize and miniaturize liver cultures**

The ability to control tissue architecture along with cell-cell and cell-matrix interactions on the order of single cell dimensions represents another important tool in the investigation of mechanisms underlying tissue development, and ultimately, the realization of tissue-engineered systems. Semiconductor-driven microtechnology tools enable micrometer-scale control over cell adhesion, shape and multicellular interactions [85,86]. Thus, over the last decade, microtechnology tools have emerged both to probe biomedical phenomena at relevant length scales and to miniaturize and parallelize biomedical assays (e.g., DNA microarrays, microfluidics).
In the context of the liver, a photolithographic cell patterning technique (‘micropatterned co-culture’) has enabled the optimization of liver-specific functions in co-cultures via engineering of the balance between homotypic (hepatocyte-hepatocyte) and heterotypic (hepatocyte-non-parenchymal) cell-cell interactions [50]. Specifically, micropatterned co-cultures were created in which hepatocyte islands of controlled diameters were surrounded by supporting non-parenchymal cells (Fig. 46.5). This pattern was miniaturized into a multiwall format using soft lithography techniques and has resulted in micropatterned co-cultures optimized for human hepatocyte function [14]. The maintenance of hepatic functions has been shown to be highly dependent upon precise optimization of island size in systems utilizing both rat and human hepatocytes. This platform has been utilized extensively for both drug development and pathogen model systems, as discussed in detail in the next section.

As described above, several culture techniques have been explored for the formation of hepatic spheroids. These methods exhibit certain limitations, including an inability to immobilize the spheroids at defined locations and the heterogeneity of the structures, which can result in

**FIGURE 46.4**
Bioreactors for in vitro liver applications. (a) Zonation and toxicity in a hepatocyte bioreactor. Co-cultures of hepatocytes and non-parenchymal cells are created on collagen-coated glass slides and placed in a bioreactor circuit where the oxygen concentration at the inlet is held at a constant value. Depletion of oxygen by cells creates a gradient of oxygen tensions along the length of the chamber, similar to that observed in vivo. (b) Two-dimensional contour plot of the medial cross section of the reactor depicting the cell surface oxygen gradient formed with inlet pO2 of 76 mmHg and flow rate of 0.3 mL/min. (c) Bright-field images of perfused cultures treated with 15 mM acetaminophen (APAP, a hepatotoxin) and stained with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) as a measure of cell viability from five regions along the length of the bioreactor. The intensity of MTT staining is reported as relative optical density (R.O.D) values. The zonal pattern of APAP toxicity seen here is consistent with that observed in vivo [83]. (d) A zonal microbioreactor array that incorporates serpentine mixing regions and two sources is able to create a gradient of fluorescein (shown in blue on right) in an array of microbioreactors containing random co-cultures of hepatocytes and non-parenchymal cells (bottom). (e) A bilayer microfluidics device designed with a microchannel network that mimics liver vasculature so as to support the large metabolic needs of hepatocytes contained within an adjacent chamber. (Figure panels reproduced with permission from [82].)
cell necrosis at the core of large and coalesced spheroids due to depletion of oxygen and nutrients. Recently, microcontact printing, robot spotting techniques and micromolded hydrogels have been used to fabricate immobilized microarrays of hepatic cells or spheroids [41,73,87–90]. Hepatocytes in these platforms typically retain a liver-specific phenotype as assessed by the expression of liver-enriched transcription factors, secretion of albumin, and the presence of urea cycle enzymes. Microtechnological tools have also been applied to the analysis of dynamic processes related to hepatocyte biology. For example, microfluidic devices that recreate physiologically relevant fluid flows and lengths scales have been used to study drug clearance, toxicity and inflammation-mediated gene expression changes [78,91–93], and mechanically actuated, microfabricated substrates have been used to deconstruct the role of contact and short-range paracrine signals in interactions between hepatocytes and stromal cells or liver endothelial cells [53,94]. Overall, the fine spatial and temporal control of molecular signals provided by microtechnological approaches continue to reveal important mechanisms in liver biology and accelerate the development of therapeutic strategies.

Drug and disease model systems

Advances in the development of liver cell-based culture systems have begun to provide important insights into human-specific liver processes that were not previously accessible with standard in vitro models. Specifically, engineered tissue systems which promote long-term functional stabilization of human hepatocytes in vitro have enabled novel studies into drug-drug interactions and hepatocellular toxicity. For example, micropatterned co-culture-based platforms have been demonstrated to support human hepatocyte phenotypic function for several weeks, including maintenance of gene expression profiles, canalicular transport, phase I/II metabolism, and the secretion of liver-specific products [14,95]. Furthermore, drug-mediated modulation of CYP450 expression and activity, and resultant changes in toxicity profiles were observed, illustrating the utility of the platform for human ADME/Tox (adsorption, distribution, metabolism, excretion, and toxicity) applications (Table 46.3).
Several other engineered model systems incorporating 3D hepatocellular aggregates or perfusion methods have also been designed for drug screening purposes and tested with either rodent or human hepatocytes [26,68,96,97].

Engineered in vitro liver models can also facilitate studies into the behavior of pathogens that exclusively target human hepatocytes, including two such pathogens with profound global health implications, hepatitis C virus (HCV) and malaria. Early experiments examining HCV replication in vitro employed carcinoma cells stably transfected with a subgenomic viral replicon [98]. While these studies provided important information on viral replication and potential small molecule inhibitors of viral replicative enzymes, the entire viral life cycle could not be completed due to the absence of structural proteins. Furthermore, prior to 2005, there was no known viral genotype that could complete the viral life cycle in vitro. Following the identification of a genotype-2a strain of HCV responsible for fulminant hepatitis in a Japanese patient, termed JFH-1 [99], it was demonstrated that JFH-1 and a chimeric variant could complete a full viral life cycle in vitro [100]. Subsequently, recent approaches have made it possible to examine HCV infection in primary human hepatocytes [16,103,104]. In particular, the stabilization of human hepatocytes in a micropatterned co-culture platform was demonstrated to allow the recapitulation of the full viral life cycle in vitro [16] (Fig. 46.6). In this platform, human hepatocytes expressed all known entry factors for HCV and supported viral replication for several weeks, illustrating the potential of such in vitro systems for screening drug candidates that suppress HCV replication, and the ultimate identification of non-hepatotoxic anti-HCV compounds. Furthermore, a recent study demonstrating HCV infection of human iPS-derived hepatocyte-like cells establishes a foundation for the development of personalized disease models that allow the study of the impact of host genetics on viral pathogenesis [105].

In vitro culture platforms have also been explored for the study of hepatocyte infection by Plasmodium sporozoites in order to model the liver stage of malaria. To date, the primary cell

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**TABLE 46.3** Generation of major human drug metabolites by different in vitro systems [95,254]

<table>
<thead>
<tr>
<th>Excretory metabolites &gt; 10% of dose</th>
<th>In Vivo</th>
<th>Microsomesa</th>
<th>S-9b</th>
<th>Hepatocyte suspensionb</th>
<th>Micropatterned co-culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>All excretory metabolites</td>
<td>39</td>
<td>19 (49)</td>
<td>22 (56)</td>
<td>25 (64)</td>
<td>27 (69)</td>
</tr>
<tr>
<td>Metabolites arising by phase 1 reactions only</td>
<td>29</td>
<td>17 (59)</td>
<td>19 (66)</td>
<td>19 (66)</td>
<td>20 (69)</td>
</tr>
<tr>
<td>Metabolites arising by a phase 2 reaction</td>
<td>10</td>
<td>2 (30)</td>
<td>3 (30)</td>
<td>6 (60)</td>
<td>7 (70)</td>
</tr>
<tr>
<td>Metabolites that are one reaction from parent (primary)</td>
<td>16</td>
<td>12 (69)</td>
<td>11 (69)</td>
<td>12 (75)</td>
<td>13 (81)</td>
</tr>
<tr>
<td>Metabolites that are two or more reactions from parent (secondary)</td>
<td>23</td>
<td>7 (48)</td>
<td>11 (48)</td>
<td>13 (57)</td>
<td>14 (61)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Circulatory metabolites &gt; 10% of total drug-related material</th>
<th>In Vivo</th>
<th>Microsomesa</th>
<th>S-9b</th>
<th>Hepatocyte suspensionb</th>
<th>Micropatterned co-culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>All circulating metabolites</td>
<td>40</td>
<td>17 (43)</td>
<td>19 (48)</td>
<td>21 (53)</td>
<td>28 (70)</td>
</tr>
<tr>
<td>Metabolites arising by phase 1 reactions only</td>
<td>31</td>
<td>14 (52)</td>
<td>16 (52)</td>
<td>14 (45)</td>
<td>22 (71)</td>
</tr>
<tr>
<td>Metabolites arising by a phase 2 reaction</td>
<td>9</td>
<td>3 (33)</td>
<td>3 (33)</td>
<td>7 (78)</td>
<td>6 (67)</td>
</tr>
<tr>
<td>Metabolites that are one reaction from parent (primary)</td>
<td>16</td>
<td>11 (69)</td>
<td>11 (69)</td>
<td>12 (75)</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Metabolites that are two or more reactions from parent (secondary)</td>
<td>24</td>
<td>6 (25)</td>
<td>8 (33)</td>
<td>9 (38)</td>
<td>16 (57)</td>
</tr>
</tbody>
</table>
culture models that have been utilized include primary human hepatocyte monolayers and human hepatoma cells [106–108], and have provided important information regarding hepatocyte invasion and the role of SR-BI and CD81 [109], and potential targets for attenuating parasite growth [110]. Recently, micropatterned co-cultures which support long-term functional maintenance of primary human hepatocytes have been shown to recapitulate the liver stage of two human malaria species, *Plasmodium falciparum* and *Plasmodium vivax*, in vitro [111]. By enhancing the efficiency and duration of *Plasmodium* infection in vitro, such engineered hepatocyte culture platforms could form the basis for drug screening and vaccine validation assays against *Plasmodium* in an era of renewed interest and focus on global malaria eradication.

The field of hepatic tissue engineering continues to evolve towards creating an optimal microenvironment for liver cells in vitro. Overall, it is evident that many different culture conditions can preserve at least some phenotypic features of fully functional hepatocytes. Since detailed differences can exist in these model systems, current strategies have focused on selecting a platform that is appropriate for a particular application. Although much progress has been made, further work is required to obtain a more complete picture of the molecular signals that provide phenotypic stability of liver cultures. Indeed, it is likely that many pathways contribute to the differentiated state of the hepatocyte via a systems-level network. Stimulation of this network via different inputs (e.g., using different culture systems) may result in distinct signal transduction trajectories, each of which could lead to a ‘phenotypically stable’ hepatocyte. It is also possible that differences between such stabilized hepatic populations will emerge as new methods to probe phenotype and function in greater detail (e.g., via epigenetics, proteomics, metabolomics) are utilized. Future challenges, therefore, will be to identify such differences and understand them in the context of network-level (in vivo) hepatic phenotype. The development of highly functional and systems-level [112,113] in vitro liver platforms will ultimately facilitate both the clinical effectiveness of cell-based therapies as well as enable high-throughput screening of candidate drugs for liver-specific metabolism and toxicity earlier in the drug discovery pipeline and for human hepatotropic infectious diseases such as HCV and malaria.
EXTRACORPOREAL BIOARTIFICIAL LIVER DEVICES

One promising approach for cell-based therapies for liver diseases is the development of extracorporeal support devices, which would process the blood or plasma of liver failure patients. These devices are principally aimed at providing temporary support for patients suffering from acute or acute-on-chronic liver failure, to enable sufficient regeneration of the host liver tissue, or serve as a bridge to transplantation. Early extracorporeal device designs utilized primarily non-biological mechanisms such as hemoperfusion, hemodialysis, plasmapheresis, and plasma exchange \[1\] \[14\]. Hemoperfusion removes toxins, but also captures other useful metabolites, by passage of blood or plasma through a charcoal column. A modification of this approach, referred to as hemodiadsorption, reduces direct contact with charcoal components through the utilization of a flat membrane dialyzer containing charcoal and exchange resin particles \[1\] \[15\]. In general, charcoal perfusion systems have been the most extensively studied non-biological configuration, including clinical evaluation in patients with acute liver failure, although no clear survival improvement has been observed \[1\] \[16\]. More recent configurations of artificial support systems have focused on the elimination of albumin-bound toxins utilizing a method termed albumin dialysis. These devices, such as the Molecular Adsorbent Recirculating System (MARS\textsuperscript{®}) and the Prometheus platform, clear albumin-bound toxins through interaction with an albumin impregnated dialysis membrane \[1\] \[17\]. They have been shown to offer some clinical benefit by reducing plasma bile acids, bilirubin, and other albumin-bound toxins \[1\] \[18\], but additional randomized controlled trials are required to fully evaluate their effectiveness.

In order to provide a more complete array of synthetic, metabolic, and detoxification functions, which are lacking in strictly artificial support systems, biological approaches including cross hemodialysis, whole liver perfusion, and liver slice perfusion have been explored \[1\] \[16\]. While these methods have shown minor improvements for patients with acute hepatic failure, they have not demonstrated any clinically meaningful survival benefits. Consequently, substantial efforts have been placed towards the development of extracorporeal BAL devices containing hepatic cells. BAL devices can be categorized into four main types, summarized in Fig. 46.7: hollow fiber devices, flat plate and monolayer systems, perfusion bed or porous matrix devices, and suspension reactors. Broadly, several criteria have emerged as central to the design of an effective BAL device; these include issues of cell sourcing, maintenance of cell viability and hepatic functions, sufficient bidirectional mass transfer, and scalability to therapeutic levels. In this section, we discuss these issues and review recent advances in BAL device design.

**Cell sourcing**

As discussed earlier, the sourcing of hepatic cells is a fundamental challenge for all liver cell-based therapies. Most BAL devices are forced to employ xenogeneic sources (primarily porcine) or immortalized human hepatocyte cell lines \[1\] \[19\] \[122\]. Porcine hepatocytes are well studied, easily sourced, and offer a functional profile quite similar to human hepatocytes, but are limited by safety concerns, such as the possibility of transmitting pathogens like porcine endogenous retrovirus (PERV), across species. However, patients treated with porcine-derived therapies have all been negative for PERV infection \[1\] \[123\]. Human hepatocyte cell
lines, while expandable, exhibit an abnormal repertoire of liver functions, which limits their clinical impact. Primary human hepatocytes present the best functional output, but are scarce and rapidly lose viability and phenotype after procurement and isolation, although recent advances in cryopreservation, fetal cell procurement and stem cell differentiation have helped primary human cells become increasingly available [124–127]. Magnifying the cell sourcing challenge are scalability requirements for translation, which will be discussed in detail in the scale-up section of this chapter.

**Cell viability and function**

The development of effective BAL systems is dependent on the incorporation of appropriate environmental and organizational cues, which enable maximal survival and function of the hepatocellular component. Hollow fiber devices are the most common BAL design and contain hepatic cells within cartridge units similar to those utilized in hemodialysis systems [128]. The hollow fiber membranes serve as a scaffold for cell attachment and compartmentalization, although adequate nutrient transport and proper environmental stimuli are potentially limited in these configurations. Multiple modifications aimed at optimizing cellular performance have been explored. In particular, due to the enhanced function of hepatocyte spheroids relative to dispersed cells, many device configurations contain either attached or encapsulated hepatocyte aggregates [128]. For example, the original HepatAssist system previously developed by Circe Biomedical was a hollow fiber device containing microcarrier-attached porcine hepatocyte aggregates within the extracapillary space [129]. Collagen gel entrapment of hepatocyte aggregates has also been added to some hollow fiber designs to improve function, and many perfusion bed systems integrate hepatocyte aggregates within a polymeric network of pores or capillaries [120]. Furthermore, encapsulation of pre-formed hepatocyte spheroids within calcium-alginate beads has been explored as a means of promoting hepatocyte stability while simultaneously providing an immunoisolation barrier [128]. In the modular extracorporeal liver support (MELS) system (Charite, Germany) hepatocytes are aggregated in co-culture with non-parenchymal cells resulting in the formation of tissue-like organoid structures [130]. Bile canaliculi and bile duct structures as well as matrix deposition have been observed in this system, which utilized both porcine cells and primary human hepatocytes isolated from livers unsuitable for transplant [121]. Platforms based on collagen gel ‘sandwich culture’, a stable hepatocyte-only culture configuration, have also been developed recently [131]. Moreover, exposure of hepatocytes to the plasma or blood of a sick patient may necessitate alterations in hepatocyte culture conditions. Specifically, pre-conditioning with physiologic levels of insulin, lower than that present in normal culture medium, has been shown to prevent fat accumulation in hepatocytes upon exposure to plasma [132]. Additionally, the supplementation of plasma with amino acids has been demonstrated to increase albumin and urea synthesis [133].

**Mass transfer**

Bidirectional mass transfer is another primary consideration in the design of BAL systems and is required to provide vital nutrients to the incorporated cells and simultaneously allow export of therapeutic cellular products. Device configuration determines both the convective and diffusive properties of the system, thereby dictating the exchange of soluble components. In particular, diffusion resistance is often a major constraint in BAL devices. Factors commonly limiting diffusive transport are membrane structures, collagen gels, and non-viable cells. For example, semipermeable membranes are often utilized in BAL devices in order to enable selectivity in the size of exchanged factors. Inherent to most hollow fiber devices, but also utilized in some flat plate and perfusion bed systems, such membranes with a designated molecular weight cutoff act to prevent the transport of immunologic components and larger xenogeneic substances while maintaining transport of carrier proteins such as albumin. Although clearly not ideal, in order to maximize mass transfer, immunologic barriers have been eliminated from some device designs [134], with the assumption that the short duration
of contact with xenogeneic cells will result in minimal immunological complications. Perfusion bed systems allow for enhanced mass transfer due to the direct contact with the perfusing media, although fluid flow distribution is highly contingent on the type of packing material. Encapsulation of dispersed or aggregated cells represents another strategy for immunoisolation but can increase diffusion resistance [135,136]. The development of novel methods for the microencapsulation of hepatocytes is an active research area and is discussed in more detail in the implantable applications section of this chapter.

Oxygen tension is an important mediator of hepatocyte function [137–139]; thus, the improved regulation of oxygen delivery is a major goal of many recently developed BAL platforms. Strategies have included the incorporation of additional fiber compartments that carry oxygen directly into the device [140,141], and more recently, the incorporation of bovine red blood cells to the circulating culture medium [142]. Studies have confirmed that hepatocyte viability and function are more effectively maintained in devices with enhanced oxygen transport [143,144]. In contrast with other designs, flat plate geometries can be perfused in a relatively uniform manner, although this configuration may result in the exposure of cells to shear stress causing deleterious effects on cellular function [131,145–147]. Several approaches for minimizing shear stress exposure in the flat plate geometry have been explored, including the fabrication of grooved substrates for shear protection as well as the integration of adjacent channels separated by a gas-permeable membrane as a means to decouple oxygen exchange and volumetric flow rate [80,147,148]. Notably, as discussed earlier, oxygen has been implicated in the heterogeneous distribution of hepatocyte functions along the liver sinusoid, and this zonation can be recapitulated in vitro within a bioreactor system [149]. The eventual incorporation of oxygen gradients, as well as gradients of other diverse stimuli such as hormones and growth factors, into BAL designs could provide a means to more closely simulate the range of hepatocyte functions exhibited in vivo and further enhance the effectiveness of BAL devices.

Scale-up

The successful clinical implementation of any BAL device is dependent on the ability to scale the device to a level that provides effective therapy. It is estimated that such BAL devices would require approximately $1 \times 10^{10}$ hepatocytes representing roughly 10% of total liver weight [150], although distinct categories of liver disease, such as acute liver failure, end stage cirrhosis, inherited metabolic disorders, will have varied scale requirements. Hepatocyte transplantation experiments in humans as well as rat models have demonstrated some improvement in various blood parameters following the transplantation of cell numbers representing 1–10% of total liver mass [151–154]. BAL devices that have been tested clinically have used approximately $0.5 \times 10^{9}$ to $1 \times 10^{11}$ porcine hepatocytes or $4 \times 10^{10}$ C3A hepatoblastoma cells [120]. Ultimately, to achieve clinical efficacy, approaches will require a) scaled-up systems with efficient nutrient transport and b) expandable cell sources, as discussed in earlier sections of this chapter. Accordingly, increasing cartridge size and use of multiple cartridges have been utilized as a means to scale-up hollow fiber-based systems. Perfusion systems [155] or devices utilizing encapsulated cells can be scaled fairly easily to the desired size; however such expanded configurations normally present a large priming or ‘dead’ volume. Stacked plate designs have been suggested as a means of scaling-up flat plate systems, although these modifications may introduce channeling effects and heterogeneous flow distribution [114]. Overall, the development of BAL devices exhibiting therapeutic levels of function is a major challenge and modifications aimed at further improving the capacity and efficiency of these systems is a central goal in the field.

Regulation and safety

Similar to other tissue-engineering-based therapies, the regulation of BAL devices is complex, due to the hybrid nature of these systems. Currently, BAL devices are being regulated as drugs...
through the Center for Biologics Evaluation and Research of the Food and Drug Administration. The primary safety concerns for BAL systems are similar to those for other cell-based therapies, and include the escape of tumorigenic cells, immune reactions to foreign antigens and xenozoonosis. Other potential complications generally associated with extracorporeal blood treatment include hemodynamic, metabolic, and hypothermia-related abnormalities, as well as problems linked with catheterization and anticoagulation [118]. In order to explicitly prevent escape of tumorigenic cells, such as the C3A cell line used in the ELAD system, downstream filters have been added to BAL designs [119], an approach which is generally acknowledged as an adequate precautionary measure.

With regard to the utilization of porcine hepatocytes, there is some evidence for the presence of antibodies directed against porcine antigens in the serum of patients treated with BAL devices [156]. However, the clinical significance of these findings remains unclear, since high titers are not observed until one to three weeks, for IgM and IgG isotypes, respectively. These results suggest that immune rejection may not be a significant problem in the context of BAL therapy, except in cases of repetitive treatments. Still, appropriate modifications through cell sourcing or device design aimed at limiting immunologic complications would likely be important in expansion of BAL treatment options to chronic liver disease patients and patients with repetitive episodes of acute decompensation. As mentioned earlier, exposure to porcine cells can also represent a risk of xenozoonotic transmission of PERV, ubiquitous in the genome of bred pigs. PERV has been shown to infect human cell lines in vitro [157], although studies examining transmission to BAL-treated patients have not demonstrated any evidence of infection [158]. While specific transmission of PERV may not occur during the course of BAL therapy, the use of xenogeneic cells in BAL devices will always incur a note of caution.

**Ongoing clinical trials**

A number of BAL devices have been tested clinically, and the characteristics of these systems are provided in Table 46.4. Important practical issues include the use of whole blood versus plasma, and the type of anticoagulation regimen. The use of whole blood exhibits the advantage of including oxygen-containing erythrocytes; however, undesirable leukocyte activation and cell damage may arise. In contrast, perfusion of plasma prevents hematopoietic cell injury, but the solubility of oxygen in plasma devoid of oxygen carriers is quite low. Furthermore, heparin coagulation is normally used in BAL systems, although deleterious effects of heparin exposure on hepatocyte morphology and function have been suggested [159].

The design of clinical trials for BAL devices poses a significant challenge. In particular, liver failure progression is highly variable and etiology dependent. Additionally, hepatic encephalopathy, one of the major manifestations of liver failure is difficult to quantify clinically. As a result, patients in clinical trials must be randomized while still controlling for the stage at which support was initiated as well as individual etiology. Similarly, the determination of the relevant control therapy can be difficult. Ideally, in order to minimize non-specific effects of extracorporeal treatment, a non-biological control such as veno-venous dialysis would be utilized. Another challenge is the choice of clinical end point. Most clinical trials utilize end points of 30-day survival and 30-day transplant-free survival; however, trials can often be confounded by the fact that acute liver failure patients receive transplants variably depending on organ availability and the eligibility criteria in place at a given center. Furthermore, interpretation of the specific role of incorporated, live, functional hepatocytes can be complicated by the presence of non-biological adjuncts such as charcoal perfusion in some designs. A direct comparison of the effect of non-biological systems alone, dead or non-hepatocyte cells, and live hepatocytes would provide substantial insight concerning the effectiveness of the cellular components, particularly given that dead hepatocytes and non-hepatocyte cells have been shown to offer some survival benefit in various animal models of
acute liver failure [160]. Notably, the ability to more accurately assess viability and function of cells during BAL treatment would be a major advance. Such information would be crucial in determining treatment time and the potential requirement for device replacement, both important considerations due to the instability of hepatocellular function in many contexts and the demonstrated detrimental effect of plasma from liver failure patients on cultured hepatocytes [161]. Finally, even if clinical trials of current BAL devices do not prove efficacy, information obtained from these studies coupled with improvements in cell sourcing and functional stabilization will represent the foundation for the next generation of devices.

**IMPLANTABLE TECHNOLOGIES FOR LIVER THERAPIES AND MODELING**

In addition to temporary extracorporeal support, the development of cell-based therapies for liver treatment aimed at the eventual replacement of damaged or diseased tissue is an active area of investigation. In many cases, hepatocytes have been injected into animal hosts and exhibit substantial proliferative capacity as well as the ability to replace diseased tissue and correct metabolic liver deficiencies in those models [162–165]. However, the clinical efficacy of these procedures is currently limited due in part to technical hurdles in cell delivery and animal models. These limitations might be addressed in part by engineering three-dimensional liver tissue ex vivo prior to implantation. Here we detail the state-of-art in cell transplantation in the context of applicable animal models as well as in the construction and application of engineered liver tissue.
Cell transplantation and animal models

Hepatocytes have been transplanted into rodents via injection into the spleen or splenic artery, intraperitoneal space, peripheral veins, or portal vein. Cell transplantation can improve host survival in animals with acute liver failure induced both chemically and surgically as well as end stage liver failure due to cirrhosis [165–169]. Transplantation also can correct metabolic deficiencies in several models of liver-based metabolic diseases [165,166]. Despite encouraging results in cell transplantation in animal models, translation of cell transplantation to the clinic has failed to result in sustained improvements in patient outcome. These results demonstrate the need for further improvements in cell delivery (e.g., through modalities such as tissue engineering) and/or better animal model systems.

Models of acute liver failure include administration of clinically relevant toxic doses of carbon tetrachloride (CCl4) or acetaminophen, which can induce localized centrilobular necrosis, or surgical resection of two thirds of the liver in a partial hepatectomy model [170]. In these models, animals develop severe liver injury, but if animals can be kept alive for as little as 72 hours following injury, host regeneration can rapidly correct the damage. This observation is unlike the human situation, in which the repair of damage due to acute liver failure in patients has been estimated to take weeks or months [171,172]. This discrepancy could in part explain contradictory results in which animals can be ‘cured’ following acute liver failure after hepatocyte transplantation whereas humans are not; it is possible that injected hepatocytes are able to provide short-term support to animals but not the long-term replacement of hepatic function necessary for clinical therapy. Of note, parabiotic systems have demonstrated that after partial hepatectomy, factors regulating liver cell proliferation are present in the circulation [173,174]. Consequently, this model may serve as a well-controlled system to examine the importance of regenerative cues in the engraftment and proliferation of hepatic constructs implanted in extrahepatic sites.

Chronic liver failure represents a more substantial clinical burden, as outlined above. The most common animal models of experimental fibrosis are toxic damage due to CCl4 administration and bile duct ligation [175]. In a rat cirrhosis model induced by CCl4 treatment, hepatocytes transplanted into the spleen result in improved survival over controls for a period of months [176–178]. To date, hepatocyte transplantation has resulted in improvements in liver function and encephalopathy in some patients with acute and chronic liver failure, but no change in outcome and survival of these patients has been observed [179]. Engineering strategies to improve engraftment and survival in extrahepatic sites may improve the efficacy of these therapies.

Finally, hepatocytes have been transplanted into several animal models of metabolic liver diseases. In most cases, transplantation only partially corrected the genetic abnormalities. However, in the fumaryl acetoacetate hydrolase (FAH)-deficient mouse model of familial tyrosinemia [180], the albumin-uPA transgenic mouse [181], and in the transgenic mouse model of human alpha-1-antitrypsin deficiency [182], the inherited metabolic defect results in reduced survival of host hepatocytes and transplanted hepatocytes are able to engraft and replace the hepatocytes in the host liver over the course of weeks or months. These findings led to the generation of ‘chimeric’ mice, in which a substantial portion of the liver is composed of human hepatocytes (Fig. 46.8). For this reason, FAH-deficient and albumin-uPA transgenic mice are the most frequently used animal models for studying hepatocyte engraftment in host liver. Additionally, such mice promise to be useful as model systems for investigating human-specific drug metabolism and toxicity as well as infectious diseases that exhibit human tropism. The most convincing data for the therapeutic use of hepatocyte transplantation to date has been demonstrated in patients, primarily pediatric, with liver-based metabolic disorders. Patients with certain disorders such as urea cycle disorders experience greater improvement than others, but even in these cases, the function of hepatocytes deteriorates with time such that liver transplantation is typically necessary by six months [183].
In all of these animal models, a regenerative stimulation is provided by transgenic injury, partial hepatectomy, portocaval shunting, or the administration of hepatotoxic agents prior to cell transplantation. However, as evidenced by recent clinical trials that have seen limited success, these approaches are difficult to adapt to a clinical setting. Major limitations of isolated cell transplantation include the inefficient engraftment and limited survival of transplanted hepatocytes, which has been collectively reported at only 10–30% of injected cells [184]. Several studies have demonstrated methods to improve engraftment and enhance the selective proliferation of transplanted hepatocytes [185–187], although analogous to regeneration models, the clinical utility of these approaches remains to be determined. The time for engraftment and proliferation of transplanted hepatocytes also can create a

**FIGURE 46.8**

**Mice with humanized livers.** (a) FAH-positive human hepatocytes injected into the spleen of Fah−/− mice can engraft and integrate in the mouse livers, resulting in mice with “humanized” livers. (b) Human albumin can be detected in the blood of these mice. (c) Engineered human liver tissue (“ectopic liver tissue”) could also be constructed ex vivo and then implanted into mice. (d) Ectopic liver tissue can survive and function in mice that are both immunocompromised (left) and immunocompetent (right). (e) Human albumin can be detected in the blood of these mice. (f) Mice with ectopic human liver tissue can be used to identify disproportionate human drug metabolites that would not have been identified by wild-type mice. (Reproduced with permission from [15,180].)
substantial lag time (48 hours in one study[152]) before clinical benefit is observed, which
could restrict the utility of cell transplantation for certain clinical conditions, such as fulminant
hepatic failure.

In summary, clinical trials of hepatocyte transplantation have recently demonstrated long-term
safety, but donor hepatocyte engraftment and restoration of failing host livers has not been
adequate to obviate the need for organ transplantation[188–190]. Additionally, only partial
correction of metabolic disorders has been accomplished to date in the clinic[188–190].
Future studies that improve cell delivery, survival, and engraftment as well as reduce the time
required for integration of the grafted cells with the host could greatly improve the effective-
ness of cell-based therapies and animal model systems.

**Implantable engineered tissue for humanized mouse models**

Implantation of human engineered liver tissue into animal hosts may also provide alternative in
vivo model systems for human disease. Despite their promise as model systems for investigating
human-specific drug responses and infectious diseases with human tropism, current humanized
mouse models (e.g., FAH[−/−] and albumin-uPA models detailed above) are limited in that animals
must be both immunodeficient and exhibit significant host liver injury. Additionally, the process of
human hepatocyte injection, homing to the liver, and expansion can take weeks to months; creating
humanized mice using ‘classic’ cell transplantation is therefore tedious and time-consuming[15].
As one example of a candidate alternative, a recent study generated humanized mice by implanting
hepatocytes and supporting non-parenchymal cells within a three-dimensional hydrogel scaffold
into the intraperitoneal space of uninjured mice[15] (Fig. 46.8). The engineered human liver tissue
synthesized human liver proteins as well as human-specific drug metabolism, drug-drug interac-
tion, and drug-induced hepatocellular toxicity. The polyethylene glycol (PEG)-based engineered
tissue was shown to survive and function within immunocompetent hosts for a period of time after
implantation, suggesting that encapsulation of cells in this material system may have the potential
to delay immune rejection and enable studies that require both human liver systems and intact
immune processes.

**Implantable therapeutic engineered liver tissue**

The development of implantable engineered hepatic tissue is a promising strategy for the
treatment of liver disease due to its potential to mitigate the limitations in current cell
transplantation strategies, including inefficient seeding and engraftment, poor long-term
hepatocyte survival, a required donor cell repopulation advantage, and the inherent lag phase
before clinical benefit is attained[165,191]. Implantable engineered hepatic tissues are
typically fabricated by immobilizing or encapsulating hepatic cells in biomaterial scaffolds
in conjunction with strategies to optimize hepatocyte survival and function, leading to the
generation of liver-like tissue in vitro prior to in vivo implantation.

**Design criteria for implantable systems**

To achieve therapeutic levels of liver function to treat liver failure, the development of engi-
nereed hepatic tissues that contain high densities of stable and functional hepatocytes with
efficient transport of nutrients and secreted therapeutic factors is necessary. Furthermore,
integration of the engineered tissue with the host upon implantation is critical in ensuring
its long-term survival. The potential tunability of engineered implantable systems offers
attractive prospects for the optimization of hepatocyte survival and function as well as sub-
sequent host integration. Scaffold parameters that are customizable include porosity, me-
chanical and chemical properties, and three-dimensional architecture. Additionally, relevant
microenvironmental cues like paracrine and juxtacrine cell-cell interactions, cell-matrix in-
teractions and soluble factors can be incorporated into implantable engineered hepatic tissue
by translating either biological or biomimetic strategies from *in vitro* culture models so as to recapitulate important aspects of the *in vivo* hepatocyte microenvironment.

**Natural scaffold chemistry and modifications**

A wide variety of naturally-derived material scaffolds have been explored for liver tissue-engineering applications, including materials like collagen, peptides, fibrin, alginate, chitosan, hyaluronic acid, cellulose, decellularized liver matrix and composites of these [70,192–197] (Table 46.5). The choice of material determines the physicochemical and biological properties of the scaffold. For example, early efforts in developing implantable hepatic constructs utilized collagen-coated dextran microcarriers that enabled hepatocyte attachment since hepatocytes are known to be anchorage-dependent cells. The intraperitoneal transplantation of these hepatocyte-attached microcarriers resulted in successful replacement of liver functions in two different rodent models of genetic liver disorders [198]. Subsequently, collagen-coated or peptide-modified cellulose [196,199], gelatin [200], and gelatin-chitosan composite [201] microcarrier chemistries have also been explored for their capacity to promote hepatocyte attachment. On the other hand, materials that are poorly cell adhesive like alginate [70] have been exploited for their utility in promoting hepatocyte-hepatocyte aggregation (e.g., spheroid formation) and hence hepatocyte stabilization within these scaffolds. Collectively, the size of engineered tissues created by these approaches is limited by oxygen and nutrient diffusion to only a few hundred microns in thickness. To address this constraint, recent work has sought to use decellularized whole organ tissue as a matrix for liver tissue engineering (Fig. 46.9). The decellularization process removes cells from donor tissues but preserves the structural and functional characteristics of much of the tissue microarchitecture and vasculature of the underlying extracellular matrix. Decellularized liver matrices can be seeded with hepatocytes and vascular cells, exhibit liver-specific functions, and survive after transplantation into rodents [197,202]. Future work in this area will likely focus on improving cell seeding protocols, which to date have achieved only 20–40% of endogenous liver mass after recellularization, as well as co-seeding with a both hepatocytes and the various non-parenchymal cell types found in liver (e.g., stellate cells, Kupffer cells). In general, the advantages of biologically-derived materials include their biocompatibility, naturally occurring cell adhesive moieties, and, in the case of decellularization, native architectural presentation of extracellular matrix molecules.

**Synthetic scaffold chemistry**

In contrast to biologically-derived material systems, synthetic materials enable precisely customized architecture (porosity and topography), mechanical and chemical properties, and degradation modality and kinetics. Synthetic materials that have been explored for liver tissue engineering include poly(L-lactic acid) (PLLA), poly(D,L-lactide-co-glycolide) (PLGA), poly (ε-caprolactone) (PCL), and poly(ethylene glycol) (PEG) [15,71,203–207]. Polysters like PLLA and PLGA are the most common synthetic polymers utilized in the generation of porous tissue-engineering constructs. These materials are biocompatible, biodegradable, and have been used as scaffolds for hepatocyte transplantation [203,208]. A key advantage of PLGA is the potential to finely tune its degradation time due to differences in susceptibility to

<table>
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<tr>
<th>TABLE 46.5 Scaffolds utilized for hepatocellular constructs</th>
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Hydrolysis of the ester groups of its monomeric components (lactic acid and glycolic acid). However, the accumulation of hydrolytic degradation products has been shown to produce an acidic environment within the scaffold which initiates peptide degradation and stimulates inflammation, which may affect hepatocyte function [209]. Consequently, as alternatives to macroporous scaffold systems, approaches aimed at the efficient and homogeneous encapsulation of hepatocytes within a fully 3D structure have been explored. In particular, hydrogels that exhibit high water content and thus similar mechanical properties to tissues are widely utilized for various tissue-engineering applications including hepatocellular platforms. Synthetic, PEG-based hydrogels have been increasingly utilized in liver tissue-engineering applications due to their high water content, hydrophilicity, resistance to protein adsorption, biocompatibility, ease of chemical modification, and the ability to be polymerized in the presence of cells, thereby enabling the fabrication of 3D networks with uniform cellular distribution [210]. PEG-based hydrogels have been used for the encapsulation of diverse cell types, including immortalized and primary hepatocytes and hepatoblastoma cell lines [15,206,207]. The encapsulation of primary hepatocytes requires distinct material modifications (e.g., 10% w/v polyethylene glycol (PEG) hydrogel, inclusion of RGD adhesive motifs) as detailed below, as well as, analogous to 2D co-culture systems, the inclusion of non-parenchymal supporting cell types such as fibroblasts and endothelial cells [207].

**Modifications in scaffold chemistry**

The relatively inert nature of synthetic scaffolds allows for the controlled incorporation of chemical/polymer moieties or biologically active factors to regulate different aspects of cellular function. Chemical modifications like oxygen plasma treatment or alkali hydrolysis of PLGA [211,212], or the incorporation of polymers like poly(vinyl alcohol) (PVA) or poly(N-p-vinylbenzyl-4-O-β-D-galactopyranosyl-D-glucoamide) (PVLA) into poly(lactic-co-glycolic acid) (PLGA) or poly-l-lactide acid (PLLA) scaffolds [203,213,214] have improved hepatocyte adhesion by modulating the hydrophilicity of the scaffold surface [215]. Biological factors may include whole biomolecules or short bioactive peptides. Whole biomolecules are typically incorporated by non-specific adsorption of extracellular matrix molecules like collagen, laminin or fibronectin [211,216] and covalent conjugation of sugar molecules like heparin [217,218], galactose [71,219], lactose [217] or fructose [220], or growth factors like EGF [221]. Alternatively, short bioadhesive peptides that interact with cell surface integrin receptors have been extensively utilized to promote hepatocyte attachment in synthetic scaffolds.
For example, conjugation of the RGD peptide to PLLA has been shown to enhance hepatocyte attachment [222], whereas RGD modification significantly improved the stability of long-term hepatocyte function in PEG hydrogels [15,207]. The additional incorporation of adhesive peptides that bind other integrins may serve as a way to further modulate and enhance hepatocyte function within synthetic polymer substrates. Moreover, although not yet applied to hepatocellular systems, the integration of matrix metalloproteinase-sensitive peptide sequences into hydrogel networks as degradable linkages has been shown to enable cell-mediated remodeling of the gel [223–225]. The capacity to modify biomaterial scaffold chemistry through the introduction of biologically active factors will likely enable the finely tuned regulation of cell function and interactions with host tissues important for implantable systems.

**Porosity**

A common feature of many implantable tissue-engineering approaches is the use of porous scaffolds that provide mechanical support often in conjunction with cues for growth and morphogenesis. Collagen sponges, various alginate and chitosan composites, and PLGA are the most commonly used porous scaffolds for hepatocyte culture, and are generally synthesized using freeze-dry or gas-foaming techniques. Pore size has been found to regulate cell spreading and cell-cell interactions, both of which can influence hepatocyte functions [192], and may also influence angiogenesis and tissue ingrowth [226]. Porous, acellular scaffolds are normally seeded using gravity or centrifugal forces, capillary action, convective flow, or through cellular recruitment with chemokines, but hepatocyte seeding is generally heterogeneous in these scaffolds [227,228].

**Controlling 3D architecture and cellular organization**

Another approach to improving the functionality of tissue-engineered constructs is to more closely mimic in vivo microarchitecture by generating scaffolds with a highly defined material and cellular architecture, which would provide better control over the 3D environment at the microscale.

A range of rapid prototyping and patterning strategies have been developed for polymers using multiple modes of assembly including fabrication using heat, light, adhesives, or molding, and these techniques have been extensively reviewed elsewhere [229]. For example, 3D printing with adhesives combined with particulate leaching has been utilized to generate porous PLGA scaffolds for hepatocyte attachment [230], and microstructured ceramic [231] and silicon scaffolds [232,233] have been proposed as platforms for hepatocyte culture. Furthermore, molding and microsyringe deposition have been demonstrated to be robust methods for fabricating specified 3D PLGA structures towards the integration into implantable systems [234].

Microfabrication techniques have similarly been employed for the generation of patterned cellular hydrogel constructs. For instance, microfluidic molding has been used to form biological gels containing cells into various patterns [235]. In addition, syringe deposition in conjunction with micropositioning was recently illustrated as a means to generate patterned gelatin hydrogels containing hepatocytes [236]. Patterning of synthetic hydrogel systems has also recently been explored. Specifically, the photopolymerization property of PEG hydrogels enables the adaptation of photolithographic techniques to generate patterned hydrogel networks. In this process, patterned masks printed on transparencies act to localize the UV (ultraviolet) exposure of the pre-polymer solution, and thus, dictate the structure of the resultant hydrogel. The major advantages of photolithography-based techniques for patterning of hydrogel structures are its simplicity and flexibility. Photopatterning has been employed to surface pattern biological factors [237], produce hydrogel structures with a range of sizes and shapes [238,239], as well as build multilayer cellular networks [240,241].
Consequently, hydrogel photopatterning technology is ideally suited for the regulation of scaffold architecture at the multiple length scales required for implantable hepatocellular constructs. As a demonstration of these capabilities, photopatterning of PEG hydrogels was utilized to generate hepatocyte/fibroblast co-culture hydrogels with a defined 3D branched network, resulting in improved hepatocyte viability and functions under perfusion [206]. More recently, a ‘bottom-up’ approach for fabricating multicellular tissue constructs utilizing DNA-templated assembly of 3D cell-laden hydrogel microtissues demonstrates robust patterning of cellular hydrogel constructs containing numerous cell types [242]. Also, the additional combination of photopatterning with dielectrophoresis-mediated cell patterning enabled the construction of hepatocellular hydrogel structures organized at the cellular scale (Fig. 46.8). Overall, the ability to dictate scaffold architecture coupled with other advances in scaffold material properties, chemistries, and the incorporation of bioactive elements will serve as the foundation for the future development of improved tissue-engineered liver constructs that can be customized spatially, physically, and chemically.

**Host interactions**

Further challenges in the design of therapeutic implantable liver devices are more specifically associated with the interactions with the host environment. These include issues related to vascularization, remodeling, the biliary system, and immunologic considerations. Notably, a significant challenge for the design of implantable liver constructs is the need to overcome transport limitations within the grafted construct due to the lack of functional vasculature. Within the normal liver environment, hepatocytes are supplied by an extensive sinusoidal vasculature with minimal extracellular matrix and a lining of fenestrated (sinusoidal) endothelial cells. Together these features allow for the efficient transport of nutrients to the highly metabolic hepatocytes. Strategies to incorporate vasculature into engineered constructs include the microfabrication of vascular units with accompanying surgical anastomosis during implantation [232,243]. For example, polymer molding using microetched silicon has been shown to generate extensive channel networks with capillary dimensions [244]. The incorporation of angiogenic factors within the implanted scaffolds has also been explored. Specifically, integration of cytokines important in angiogenesis, such as VEGF [208], basic fibroblast growth factor (bFGF) [245], and Vascular endothelial growth factor (VEGF) in combination with Platelet-derived growth factor (PDGF) [246], has been shown to promote the recruitment of host vasculature to implanted constructs. Furthermore, preimplantation of VEGF releasing alginate scaffolds prior to hepatocyte seeding was demonstrated to enhance capillary density and improve engraftment [247].

In addition to interactions with the vasculature, integration with other aspects of host tissue will constitute important future design parameters. For instance, incorporation of hydrolytic or protease-sensitive domains into hepatocellular hydrogel constructs could enable the degradation of these systems following implantation. Of note, liver regeneration proceeds in conjunction with a distinctive array of remodeling processes such as protease expression and extracellular matrix deposition. Interfacing with these features could provide a mechanism for the efficient integration of implantable constructs. Similarly to whole liver or cell transplantation, the host immune response following the transplant of tissue-engineered constructs is also a major consideration. Immunosuppressive treatments will likely play an important role in initial therapies, although stem cell-based approaches hold the promise of implantable systems with autologous cells. Furthermore, harnessing the liver’s unique ability to induce antigen-specific tolerance [248] could potentially represent another means for improving the acceptance of engineered grafts. Finally, incorporation of excretory functions associated with the biliary system will ultimately be required in future designs. Towards this end, current studies are focused on the development of *in vitro* models which exhibit biliary morphogenesis and recapitulate appropriate polarization and bile canaliculi organization [249–251], as well as platforms for the engineering of artificial bile duct structures [252].
CONCLUSION

Although many challenges remain for the improvement of tissue-engineered liver therapies, substantial progress has been made towards achieving a thorough understanding of the necessary components. The parallel development of highly functional in vitro systems as well as extracorporeal and implantable therapeutic devices is based on contributions from diverse disciplines including regenerative medicine, developmental biology, transplant medicine, and bioengineering. In particular, novel technologies such as hydrogel chemistries, high-throughput platforms, and microfabrication techniques represent enabling tools for investigating the critical role of the microenvironment in liver function and, subsequently, the development of structurally complex and clinically effective engineered liver systems.

References


CHAPTER 46
Hepatic Tissue Engineering


PART 11
Gastrointestinal System


