Advanced Drug Delivery Reviews 62 (2010) 814–826

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Cell-delivery therapeutics for liver regeneration ☆

Wenxia Zhang [a,1], Lisa Tucker-Kellogg [b,c,d,1], B.C. Narmada [e], Lakshmi Venkatraman [l], Shi Chang [a,f], Yin Lu [c], Nancy Tan [g], Jacob K. White [c,i], Ruirui Jia [a], Sourav Saha Bhowmick [c,j], Shali Shen [a], C. Forbes Dewey Jr. [c,h], Hanry Yu [a,c,d,e,k,l,*]

[a] Department of Physiology, National University of Singapore 117597, Singapore
[b] Department of Computer Science, National University of Singapore, 117417, Singapore
[c] Singapore-MIT Alliance, Computation and Systems Biology Program, E4-04-10, 4 Engineering Drive 3, 117576, Singapore
[d] Centre for Mechanobiology, Temasek Laboratories, National University of Singapore, 5A Engineering Drive 1, 117411, Singapore
[e] NUS Graduate School for Integrative Sciences, 28 Medical Drive, 117456, Singapore
[f] Department of General Surgery, Xiangya Hospital, Central-South University, Changsha, Hunan, 410008, China
[g] Gleneagles Medical Centre, 6 Napier Road, 258499, Singapore
[h] Department of Mechanical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA, 02139, USA
[i] Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA, 02139, USA
[j] School of Computer Engineering, Nanyang Technological University, Nanyang Avenue, 639798, Singapore
[k] Institute of Bioengineering and Nanotechnology, A*STAR, 138669, Singapore
[l] Singapore-MIT Alliance for Research and Technology, S16-04-15, 3 Science Drive 2, 117543, Singapore

ARTICLE INFO

Article history:
Received 19 November 2009
Accepted 24 February 2010
Available online 1 March 2010

Keywords:
Cell transplantation
Liver regeneration
Hepatocytes
Stem cells
Xenogenic cells
Delivery vehicles
Scaffolds
Encapsulation
Systems Biology

ABSTRACT

For acute, chronic, or hereditary diseases of the liver, cell transplantation therapies can stimulate liver regeneration or serve as a bridge until liver transplantation can be performed. Recently, fetal hepatocytes, stem cells, liver progenitor cells, or other primitive and proliferative cell types have been employed for cell transplantation therapies, in an effort to improve the survival, proliferation, and engraftment of the transplanted cells. Reviewing earlier studies, which achieved success by transplanting mature hepatocytes, we propose that there is a switch-like regulation of liver regeneration that changes state according to a stimulus threshold of extracellular influences such as cytokines, matrices and neighboring cells. Important determinants of a successful clinical outcome include sufficient quantities and functional levels of the transplanted cells (even for short periods to alter the environment), rather than just engraftment levels or survival durations of the exogenously transplanted cells. The relative importance of these determining factors will impact future choices of cell sources, delivery vehicles, and sites of cell transplantation to stimulate liver regeneration for patients with severe liver diseases.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Therapeutic Cell Delivery for *in situ* Regenerative Medicine".

* Corresponding author. Department of Physiology, National University of Singapore 117597, Singapore.
E-mail address: hanry_yu@nuhs.edu.sg (H. Yu).

[1] These authors contribute equally to this work.

0169-409X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2010.02.005

1. Introduction

Liver provides several vital functions including detoxification, biotransformation, excretion, protein synthesis and hormone production [1]. Liver has evolved unique regenerative properties that allow it to heal massive injuries, such as after ingesting poisonous substances. Mature liver cells can be activated to undergo rapid division, allowing the liver to regenerate to its original mass and function after 65% partial hepatectomy [1,2]. Although the liver cannot restore the anatomical architecture of entire lobes, it can renew lobules, biliary ductwork, and other small anatomical structures. Hepatocytes normally constitute 80% of liver mass, and hepatocyte proliferation may be partially responsible for the regenerative capacity of liver [1]. Liver is frequently injured by environmental toxins, infections, alcohol, etc., and most injuries resolve naturally. Acute liver failure can arise from paracetamol overdose, ingesting poisonous mushrooms, Reye syndrome, Wilson's disease, and other causes [3]. Chronic liver injury such as from hepatitis infections or alcohol abuse causes liver fibrosis leading to cirrhosis and chronic liver failure. Metabolic disorders of the liver arise from genetic defects in key proteins in hepatocytes, which cause potentially fatal deficiencies in liver functions.

The primary treatment for liver failure, particularly chronic liver failure, is to remove the source of injury, such as treating hepatitis patients with anti-viral drugs, treating parasitic infections with anthelmintics, or halting consumption of alcohol for patients with alcohol-related fibrosis [4]. For advanced life-threatening diseases, the only widely accepted treatment is liver transplantation, which often leads to immunological complications and is limited by the availability of donor organs [5]. Additional therapies used in humans include cell transplantation and extracorporeal “artificial liver” devices [6]. Clinical studies of cell transplantation have been reviewed recently [7,8]. In this article we review cell transplantation therapies for liver, with an emphasis on regeneration.

2. Shifting paradigms

The first liver cell transplantation in human [9] involved the injection of hepatocytes for the treatment of liver failure. In early studies, mature hepatocytes (fresh or cryopreserved) were the commonly used cell sources. The outcomes of many early trials were positive [10–14], but hepatocyte availability limits widespread practice. Recent cell transplantation studies focus on stem and progenitor cells that are proliferative, integrative, and plastic; and some may be immunologically privileged to facilitate long-term integration and engraftment or prolonged cell survival upon transplantation. Although the outcomes of these pre-mature cell transplantations are positive, there has not been significant improvement in the therapeutic efficacies over the earlier transplantations of mature hepatocytes [15]. The functional capacity of mature hepatocytes is probably more important in liver cell transplantation therapies than previously appreciated.

The proliferation, differentiation, secretion, and other functions of transplanted and endogenous cells are affected by their local environments [16]. Many liver diseases decrease the liver's regenerative potential and hepatocytes' proliferative capability by influencing the extra-cellular microenvironments [17,18]. For initiation of liver regeneration after partial hepatectomy, some of the key signals come from prostaglandins [19]; the termination of regeneration is characterized by increasing levels of anti-mitogenic growth factors like transforming-growth factor beta-1 (TGF-β1) [20]. Fibrotic liver has high levels of TGF-β1, which may cause hepatocyte apoptosis [21] as well as other anti-mitotic and pro-fibrotic effects. Other key molecules in the liver microenvironment that determine regenerative behavior include the mitogen hepatocyte growth factor (HGF), pro-inflammatory cytokines, and angiogenic factors such as vascular endothelial growth factor (VEGF) [22,23]. Key cellular determinants of liver diseases and environmental signals include hepatic stellate cells (HSCs) and Kupffer cells [24]. Inflammatory cytokines and HSC activation are necessary for repair and regeneration, but excessive and protracted inflammation contributes to disease and antagonizes regeneration.

Not only does the host environment affect the transplanted cells, but the secretory and signaling outputs of the transplanted cells also alter the host environment. For example, transplantation of functional hepatocytes increases the level of HGF [25,26] which is a potent trigger of hepatocyte proliferation. Hepatocytes secrete VEGF which is important for proliferation and maintenance of sinusoidal endothelial cells (SECs) [27,28]. SECs and VEGF are in turn involved in regulating

angiogenesis and fenestration, supporting the transplanted cells [29,30]. Indeed the causes and effects may be intertwined. Multiple "vicious cycles" have been hypothesized in chronic liver diseases, such as Kupffer cell activation causing more inflammatory cytokines and oxidative stress, which in turn cause more Kupffer cell activation [31]. Cell transplantation can break the vicious cycle by altering environments (Fig. 1), for example by providing more effective antioxidant defenses than the chronically stressed endogenous cells [32]. Changes in microenvironments may have contributed to the positive outcomes of many liver cell transplantation studies; and might be initiated by the strong outputs (e.g. signaling, secretion) from the transplanted hepatocytes that drastically impact the environments to stimulate endogenous hepatocyte regeneration. For example, hepatocytes are important sources of secreted proteases that cause the activation, bioavailability, and degradation of many growth factors, cytokines, and extracellular proteins that signal for cell division [18]. Indirect effects of cell transplantation via the host environment are also illustrated by the hepatocyte transplantation work of Makowka et al. [33–35], in which hepatocyte transplantation in a rat model of liver disease was found to induce endogenous regeneration of the host liver and to improve survival. In non-liver models with analogous processes of fibrosis progression, cell transplantation has also been observed to affect microenvironments [36]. Transplanting mesenchymal stem cells (MSC) into rat lungs with endotoxin-induced fibrosis caused improvement in the disease stage by decreasing the levels of inflammatory cytokines [37] despite a very low engraftment level (<5%). The cell transplantation shifted the milieu of the injured lung to be less inflammatory in the microenvironments, leading to significant improvement in disease markers.

Similarly in our own research, labeled hepatocytes transplanted into fibrotic rats disappeared quickly, with a time series (Fig. 2a) showing a steep decay in their levels after injection. However, the percent liver area with α-smooth muscle actin, a marker of fibrosis [38] (Fig. 2b) was significantly lower at 14 days after hepatocyte transplantation, compared with the untreated fibrosis control, even though the transplanted hepatocytes were no longer detectable by that time. Thus, a time-limited burst of hepatocyte activity could cause an improvement in the liver environment that lasted longer than the cells themselves. These studies suggest that engraftment efficiency and transplant survival duration are not necessarily good yardsticks for therapeutic success. Given the importance of the local environment for the success of transplantation, researchers should assess the liver environment such as measuring cytokine levels in transplant patients, as in the approach of van Poll et al. [39].

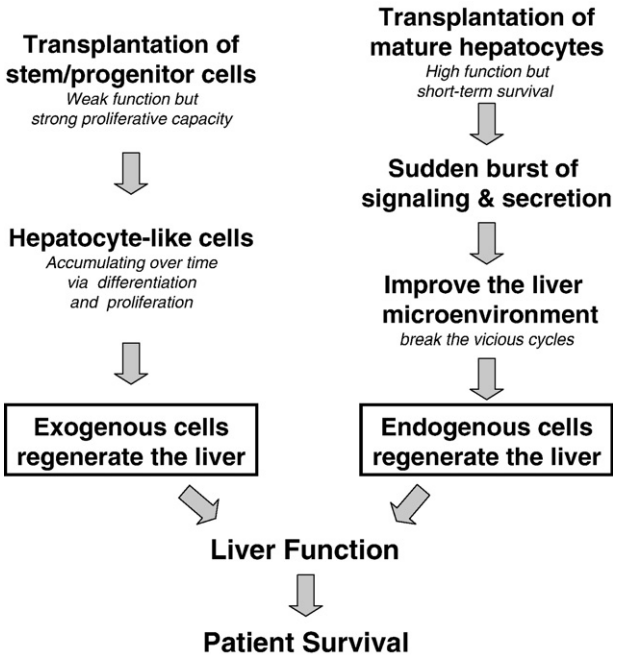

Fig. 1. Conceptual diagram of the different paradigms for understanding liver cell transplantations.

Although engraftment may not be necessary for success, the number (the "dose") of transplanted hepatocytes would have an effect on the degree of change in the environment, particularly if there is a stimulus threshold necessary for perturbing the disease state [40,41]. To investigate the activation of TGF-β1, a master regulator of fibrotic processes, we constructed a computational model of extracellular proteins involved in regulating the activation of TGF-β1. Our simulations revealed a "switch-like" pattern in the activation of this cytokine (unpublished data), suggesting the possibility of switch-like regulatory influences on the transition between fibrosis progression and regression. Some of the proteins in our simulations have been shown in cell-free experiments to exhibit a switch-like activation threshold [45]. A threshold would imply the importance of delivering a higher dose of healthy hepatocytes (a stimulus of great magnitude) into the liver. The stimulus threshold hypothesis contrasts with the previous goals of stressing the survival duration or engraftment of the transplanted cells.

Creating an environment to induce regeneration, or removing obstacles to normal healing, might only take hours to leave behind long-lasting effects. If true, this would affect choices of cell sources, delivery vehicles, sites of cell transplantation, and many future practices in liver cell transplantation therapy. Stem cells may lack potent functions whereas mature hepatocytes may encounter rejection, but hybrid approaches may achieve synergistic levels of success, particularly if mature hepatocytes can improve the host environment while stem cells integrate into the newly conducive environments.

3. Cell types transplanted for liver diseases

Hepatocytes are the source of metabolic and enzyme functions that become compromised in liver failure, and therefore are a natural cell source for restoring the missing functions. The numbers of hepatocytes needed for transplantation in human can be quite large [46], giving additional impetus to the search for alternative cell sources. Lack of donated hepatocytes is compounded by the deterioration of hepatocyte functions after storage or culturing [47]. Cells that can differentiate into hepatocytes have been of great interest. Questioning the preference for stem and progenitor cells in recent transplantation studies, a comparison study found that mature hepatocytes had better capacity [15], compared with liver progenitor cells or hepatic precursor cells derived from embryonic stem cells, for the ability to repopulate liver mass in immune-deficient mice [48]. Future studies should test this finding with a less exotic model of liver disease, along with measuring the liver environment and possible mechanisms for the relative performance of different cell transplantation therapies. The regulation of differentiation is very complex and even mature liver cell types are capable of differentiation under special conditions [10,49–52]. In this section we present a selection of clinical cases and preclinical studies illustrating the cell types used for transplantation. The relative merits of different cell types are summarized in Table 1.

3.1. Hepatocytes

As a less invasive supplementation to liver transplantation, hepatocyte transplantation has been used for the treatment of acute liver failure [53] or chronic liver failure [12]. Cell transplantation serves as a bridging method to infuse a certain number of functional hepatocytes into patients and to alleviate the symptoms while they are waiting for liver transplantation.

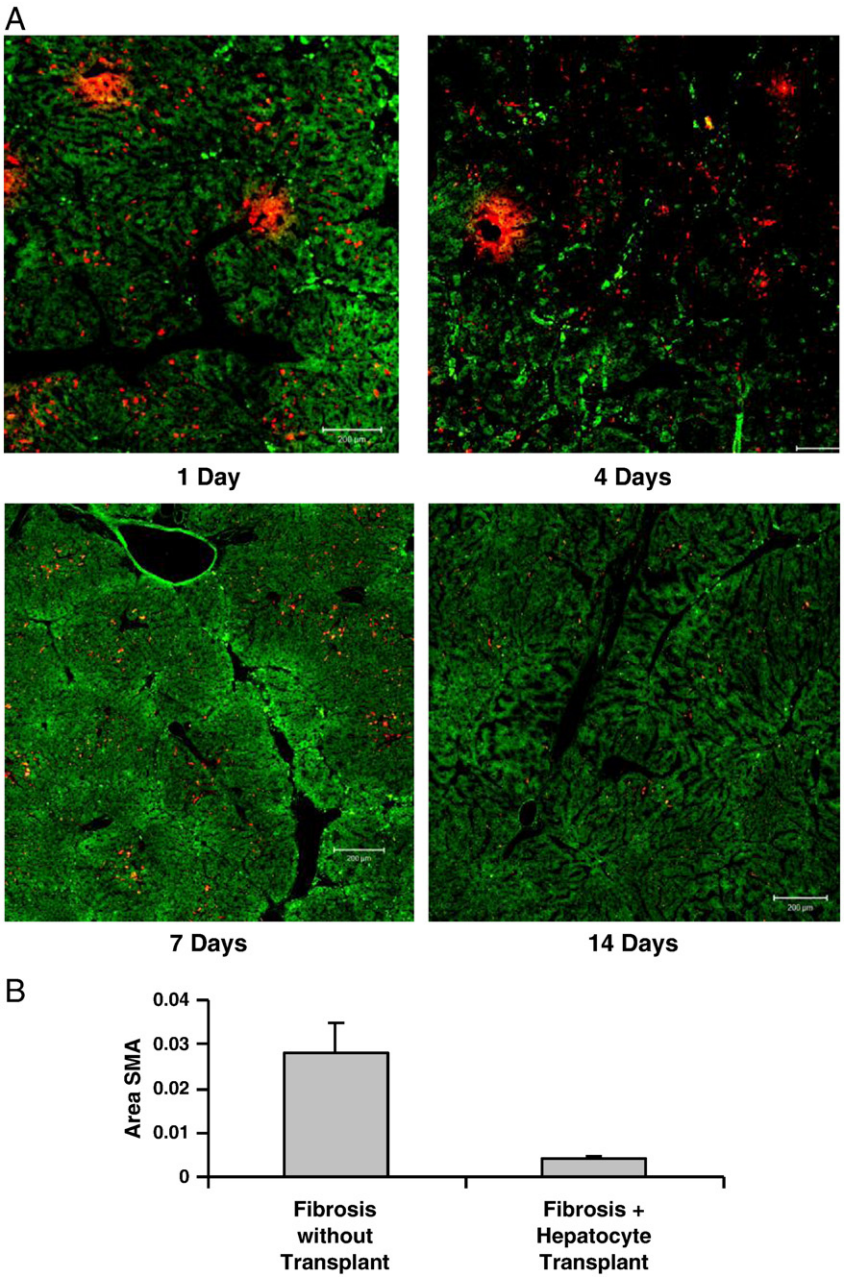

Fig. 2. Hepatocyte Transplantation into a rat model of liver fibrosis. (A) Liver tissue, imaged 1, 4, 7, and 14 days after hepatocyte transplantation, showing transplanted hepatocytes in red. (B) Percent area of liver tissue stained positive for α-smooth muscle actin ("SMA"), a marker of fibrosis [42–44], in untreated fibrotic liver, versus in fibrosis treated with hepatocyte transplantation. We injected CM-Dil labeled primary hepatocytes (5×10^7) from adult rats into the inferior pole of the spleen in the CCl_4-induced fibrotic rats. No immune-suppression was employed.

3.1.1. Acute liver failure

Hepatocyte transplantation may act as a bridge to orthotopic liver transplantation (e.g., while waiting for an organ to become available) or alternatively to trigger regeneration of the acutely-injured native liver. Several clinical trials were performed to treat fulminant hepatic failure (FHF) caused by hepatitis virus, acute toxic hepatitis, extensive liver surgery or alcohol-induced liver cirrhosis. Five patients comatose with acute liver failure received transplantation of 1.3×10^9 to 3.9×10^{10} cryopreserved hepatocytes through intrasplenic and intraportal infusion. Three patients improved afterwards, according to encephalopathy score and some liver functions. Encephalopathy is a marker of hepatocellular failure to indicate demand for organ transplantation [54] or as a predictor of survival rate. The transplanted hepatocytes were detectable in the liver or spleen of two patients 14 or 20 days post transplantation [10]. A 64-year-old woman with acute liver failure caused by mushroom poisoning was infused intraportally with 8×10^9 cryopreserved human hepatocytes, and fully recovered 12 weeks after hepatocyte transplantation without relapse [13].

Fetal hepatocytes have also been used successfully for acute liver failure. Seven patients suffering from acute liver failure received pooled human fetal hepatocytes at 60×10^6 cells/kg via intraperitoneal infusion [11]. The transplanted patients showed improved survival rate, accompanied by decreased plasma ammonia and bilirubin levels 48 h after the infusion without complications. Ammonia and bilirubin are common indicators of liver diseases. Ammonia is normally converted into urea by the liver, and its accumulation in liver diseases causes hepatic encephalopathy [55]. Bilirubin is normally excreted by the liver into bile, and elevated bilirubin is responsible for the yellow color in jaundice. A patient with acute fatty liver during

Table 1
Comparison of different cell types, delivery vehicles, and sites for cell transplantation.

	Impact on microenvironment stimulating native liver regeneration	Engraftment capability
Cell types		
1. Human hepatocytes (cryopreserved, freshly isolated, or fetal hepatocytes)	+++ Maintain metabolic and enzyme functions	• Poor engraftment
2. Stem cells (liver progenitor cells, embryonic stem cells, adipose-derived stem cells, umbilical mesenchymal stem cells, fetal liver progenitor cells, or bone marrow-derived stem cells)	• Less specialized functions	+++ Highly proliferative, integrative, and plastic; Possible long-term integration and engraftment, or prolonged survival
3. Xenogenic hepatocytes	+ Maintain metabolic and enzyme functions	• Poor engraftment; Illicit immune responses
4. Modified cells (genetic modified cells, immortalized hepatocytes, or protected from apoptosis)	+ Less specialized activity; Weaker influence on environment	+ Death resistance allows for long term survival of the transplant
Delivery vehicles		
1. Scaffolds (collagen-based scaffolds, galactose-based scaffolds, or hydrogels)	++ More stabilized metabolic functions	++ Enhanced cell attachment
2. Three-dimensional microenvironment (ECM and encapsulation)	++ Better support to cells; Better mimics *in vivo* conditions; Improved liver specific functions	++ Encapsulation reduces immune response
3. Vascularization	++ Increased blood supply and nutrients access	+ Improved cell engraftment
Sites		
1. Liver	+++ Better cell–cell interactions; Better cell–ECM interactions; Very high impact of the transplanted cells owing to proximity	+ Mediocre engraftment owing to highly vascularized nature
2. Spleen	++ Good supply of blood and nutrients; Easy access of the transplant's effects to the injured liver; Possible translocation to hepatic sinusoids	++ Very good engraftment; Hepatization observed in certain cases
3. Kidney capsule	• Long distance between injection site and target site reduces the therapeutic effect of the transplant	++ Enhanced cell survival when delivered with ECM like structures; Reduced immune response
4. Peritoneum and fat pad	• Long distance between injection site and target site reduces the therapeutic effect of the transplant; Less specialized function	+ Allows engraftment of large number of cells

pregnancy who exhibited grade IV encephalopathy for 3 days after delivery was infused with 3×10^8 human fetal hepatocytes intraperitoneally [56] and she regained consciousness within 24 h and recovered within 7 days. It is not known how long the transplanted cells survived in these patients but the single dose of transplanted hepatocytes was able to improve short-term liver functions significantly for patients with acute liver failure.

3.1.2. Chronic liver failure

The first clinical trial of hepatocyte auto-transplantation was carried out on 10 patients in 1992 [12]. The recipients' native cirrhotic left lateral liver segment was used as the cell source for hepatocyte transplantation and there was improved encephalopathy in two of the patients. In another case a 52-year-old with end-stage liver disease was infused with 2.2×10^7 cryopreserved hepatocytes into the spleen as a "bridging" technique to sustain the patient until a donor organ could become available [14]. Orthotopic whole liver transplant was performed two days after cell transplantation, and the patient fully recovered with normal liver conditions for 3 years. Another patient with end-stage liver disease and grade III encephalopathy was infused with 7.5×10^6 cryopreserved hepatocytes. The patient showed improvement in encephalopathy and plasma ammonia after cell transplantation [14]. Among seven adult cirrhotic patients with medically advanced uncontrolled encephalopathy who underwent single intrasplenic hepatocyte allo-transplantation, one patient with hepatitis C virus (HCV)-induced cirrhosis had evidence of transplanted hepatocytes forming cord like structures with normal tight cellular junctions in the spleen by day 2 post infusion [14]. Hepatocyte transplantation was performed in 3 patients with alcoholic cirrhosis in 1996 and all of them were alive in 2000 [57]. Also, for a patient with acute chronic alpha 1 anti-trypsin deficient liver disease, hepatocyte transplantation caused some improvement in symptoms and served as a bridge to liver transplantation [58].

In summary, hepatocyte transplantation has achieved considerable success in clinical settings in the past two decades and has been successfully used as a bridging method for patients with acute or chronic liver failure while waiting for liver transplantation. The availability of hepatocytes for cell transplantation becomes a limiting factor.

3.2. Stem or progenitor cells

Although hepatocytes suitable for transplantation may be obtained from donated livers, such sources are often not readily available. Researchers are working on alternative cell sources from stem cells, as they can be expanded and differentiated *in vitro* or *in vivo* [59,60]. Studies on transplantation of stem or progenitor cell sources have involved liver progenitor cells (oval cells), embryonic stem (ES) cells, multi-potent adipose tissue mesenchymal stem cells, bone marrow cells, fetal liver progenitor cells, and recently, induced pluripotent stem

(iPS) cells. Most studies have been carried out in immune-deficient animals to reduce rejection and to increase engraftment [61].

3.2.1. Liver progenitor cells (oval cells)

During chronic or acute liver injury, hepatocyte proliferation is inhibited and oval cells appear in the portal region of the lobule to facilitate liver regeneration [62]. Oval cells express α-fetoprotein (the marker of early hepatic lineage) [63,64], cytokeratin-19 (CK-19, the marker of biliary epithelium) [63,64] and albumin (the marker of mature hepatocytes) [64]. They were found to proliferate in recipient rats [63] and mice [64], showing that oval cells are intra-hepatic progenitor cells and can differentiate into mature hepatocytes and bile duct epithelial cells. Transplanted oval cells have shown impressive proliferation and engraftment capacity in non-fibrotic models of liver injury, with 90% of the hepatocytes in the recipient liver replaced by donor oval cells [65]. Further work showed that intra-hepatic injection of rat oval cells to Wistar rats with FHF could significantly increase the survival rate [66]. Given their high proliferative capacity and easier maintenance, oval cells have been considered as an alternative to primary mature hepatocytes for transplantation.

3.2.2. Embryonic stem cells

ES cells could potentially differentiate into hepatocyte-like cells [48]. *In vitro* studies have shown that murine pALB-EGFP/ES and human ES cells in cultures with growth factors could differentiate into hepatocyte-like cells [67,68]. After intravenous transplantation of murine ES cell-derived hepatic cells to the spleen or injured liver of three recipient mice, the onset of liver fibrosis was significantly suppressed and liver functions and survival were improved [68,69]. Human ES cells transplanted to the spleens of severe combined immunodeficiency (SCID) mice were also able to integrate into the injured liver [67]. ES cell studies remain at a preclinical stage because of the risk of teratomas forming from undifferentiated ES cells, but many current studies are pursuing ES cell therapies.

3.2.3. Adipose-derived stem cells (multi-potent adipose tissue mesenchymal stem cells)

In vitro-generated multi-potent adipose tissue mesenchymal stem cells ("adipose-derived stem cells") have a hepatic predisposition [70]. Transplanted adipose-derived stem cells through tail vein injection were able to differentiate into hepatocytes in BALB/c nude mice with CCl_4-induced liver injury and were able to function like human mature hepatocytes [71].

3.2.4. Umbilical mesenchymal stem cells

Injection of 5×10^5 human umbilical mesenchymal stem cells to the right lobe of rat liver with induced fibrosis was able to suppress liver fibrosis [72] even though the engrafted umbilical stem cells did not differentiate into functional hepatocytes. The authors suggested that the undifferentiated umbilical stem cells could secret certain cytokines, such as human cutaneous T cell-attracting chemokine, leukemia inhibitory factor, and prolactin, which may help to maintain liver functions and stimulate endogenous liver regeneration. Even if the transplanted pre-mature cells remain in a stem-like state with minimal secretory and immunological signaling, their functional contributions may nonetheless serve to alter the host environment significantly enough to improve the therapeutic outcome.

3.2.5. Fetal liver progenitor cells

Multiple studies [73–75] have found fetal liver progenitor cells capable of repopulating liver and differentiating into hepatocytes after partial hepatectomy in rodents. The signaling factors in post-hepatectomized liver are dramatically altered towards promoting hepatocyte proliferation, with significantly elevated levels of hepatocyte growth factor [76]. For example, a healthy liver can regenerate to 100% of the original mass after 60% hepatectomy, but a cirrhotic liver is sometimes unable to regenerate. The ability of fetal liver progenitor cells to differentiate into hepatocytes under hepatectomy conditions may motivate future investigation of whether fetal liver progenitor cells would also have therapeutic potential for other liver conditions that provide a less mitotic environment.

3.2.6. Bone marrow-derived stem cells

Murine bone marrow stem cells transplanted through tail vein injection could trans-differentiate into hepatocyte-like cells in recipient non-obese diabetic (NOD)/SCID mouse livers [77]. Similar findings after bone marrow cell transplantation to rodents have also been reported [39,78,79]. A clinical trial was performed on humans with bone marrow stem cells, using sex chromosomes to identify the transplanted cells from donors of the opposite gender [80]. The evidence of complete hematopoietic donor chimerism was confirmed [81], with the highest levels of bone marrow-derived hepatocytes in patients with severe liver diseases [82], indicating tissue damage may enhance bone marrow cells to engraft as hepatocytes. Bone marrow-derived stem cells have been particularly successful at integrating into the liver and trans-differentiating into hepatocytes.

3.2.7. Induced pluripotent stem cells

iPS technology may provide alternative cell sources of cell transplantation because iPS cells posses the characteristics of ES cells and the capability of proliferation and differentiation into multiple cell types. Transplantation of endothelial progenitor cells differentiated from iPS cells into the mouse liver with hemophilia A disease was able to improve the bleeding syndrome and the survival rate [83], indicating iPS cells might be used for human genetic disorders in the future.

The relative merits of different types of stem cells require further studies in the context of the chemical and mechanical cues in the extra-cellular microenvironments of the normal and disease states. One interesting perspective is how matrix rigidity would affect the stem or progenitor cell sources by presenting different mechanical signals directing differentiation [84–86]. The pathological stiffness of fibrotic liver may have a significant impact on the signals for stem or progenitor cell differentiation. Stem cells accustomed to a relatively soft matrix might, when presented with the stiff matrix of fibrotic liver, perceive an environment inappropriate for hepatocyte differentiation. In contrast, stem cells coming from a rigid context (e.g., bone) might perceive the fibrotic matrix as relatively soft and permissive for differentiation into hepatocytes. The role of matrix elasticity and adhesiveness for stem cell differentiation needs to be considered in the context of the fibrotic processes and matrix abnormalities that routinely occur in liver diseases. Likewise, chemical gradients and differences between the source environments where the stem or progenitor cells are isolated, and the transplantation host environments would greatly affect the effectiveness of transplantable cells to promote liver regeneration.

3.3. Xenogenic hepatocytes

Animal sources have been investigated because of the scarcity of suitable human cell sources. Pig is a commonly considered donor species for xenogenic sources of hepatocytes [1,87–89]. Porcine hepatocytes have been used in bio-artificial liver assist devices (BLAD) [1] and transplanted to monkey and rat [87,88] with some promising results. For transplantation into human, these xenogenic cells remain controversial due to the risks associated with animal viruses.

3.3.1. Pig to human

An extracorporeal porcine hepatocyte-based BLAD was employed in patients with acute liver failure leading to significantly improved patient survival [1]. Humanized (transgenic) pig liver [90] developed

to reduce the acute immune-rejection are promising cell sources that might partially alleviate the shortage of human hepatocytes for extracorporeal devices or cell transplantations.

3.3.2. Pig to monkey

$1–2\times10^9$ hepatocytes from outbred swine infused into the spleens of cynomolgus monkeys could function for more than 80 days and the xeno-transplanted hepatocytes could function for more than 253 days after re-transplantation [89]. The humoral immune response did not affect the survival of the transplanted porcine cells significantly.

3.3.3. Pig to rat

Transplantation of 5×10^7 porcine hepatocytes to cirrhotic rats can restore metabolic functions and improve survival rate [88]. Although there was immune response to the engrafted hepatocytes, transplanted porcine hepatocytes functioned well in cirrhotic rats for a period of 4 weeks without immune-suppression. A second transplantation was also successful with the assistance of the immune-suppression drug, FK506. Without immune-suppression, intraperitoneally transplanted encapsulated porcine hepatocytes could maintain liver functions for at least 15 days in rats and pigs [87].

3.4. Modified cells

Transplanted cells must survive in the hostile environment presented to them *in vivo* and still be able to function in keeping with high metabolic demands. Recent work tackles these concerns by modifying cells, either genetically or by using biomaterials (described in section 4) to enhance the delivery and sustain the therapeutic efficacy over a longer period of time.

3.4.1. Immortalized hepatocytes

A differentiated cell line was developed using normal primary adult human hepatocytes with retroviral transfer of an immortalizing gene that can later be excised [91]. The immortalized cells helped to stabilize liver functions after intrasplenic transplantation to rats with 90% hepatectomy. Another approach is the use of conditionally immortalized hepatocytes which have the potential to rapidly proliferate *in vitro* but which are engineered to avoid excessive proliferation or tumorogenesis either by apoptosing or by entering a quiescent stage after transplantation *in vivo*. For instance, thermolabile mutant simian virus 40 T antigen (SV40ts) allows *in vitro* proliferation of transfected hepatocytes at 33 °C [92], but these cells do not proliferate *in vivo* under non-permissive temperatures.

3.4.2. Protection from apoptosis

To enable preferential proliferation of the transplanted hepatocytes over the host liver's cells in gene therapy trials, Fas ligand (FasL) or irradiation were employed specifically for inducing apoptosis in the host hepatocytes, to create additional survival advantages for the transplanted hepatocytes that carried the UDP-glucoronosyltransferase (UGT1A1) gene [93]. Other strategies have been devised to reverse the rendered protection once the desired action is complete. For example, attaching a herpes simplex virus thymidine kinase gene to the SV40ts enabled the specific killing of the transplanted hepatocytes [94]. The Bcl-2 gene has also been used for giving transplanted cells protection from apoptosis. Transplantation of rat primary hepatocytes transfected with Bcl-2 gene resulted in a drastic improvement in survival rate compared to the control group in rats [95,96].

In summary, cell sources for transplantation have shifted in recent years towards progenitor cells, stem cells, and other undifferentiated cells with greater replicative potential. These cell types have many advantages, but provide less differentiated functions than the mature hepatocytes, and may provide a weaker influence towards improving the native liver environment. Mature hepatocytes provide a greater magnitude of functional improvement, even if it occurs over a short period of time, and future work can examine more thoroughly the long-term consequences of short-term perturbations. The relative benefits of various cell types should therefore be measured in terms of the clinical outcomes, rather than being presumed to correlate with surrogate markers such as cell survival and engraftment levels.

4. Delivery vehicles and non-genetic modifications to transplanted cells

The function and therapeutic potential of transplanted cells can be enhanced and sustained by delivering them in an appropriate manner. This may involve presenting the transplant with the right ECM substrates that enhance the cells' functions, engraftment and survival; creating 3D microenvironments that better mimic the normal *in vivo* conditions; or encapsulating the cells to partially isolate them from the potentially hostile *in vivo* microenvironments in the lesions. In addition, other non-genetic techniques have also been applied to enhance the engraftment efficiency and survival of the transplanted cells. A common technique is allowing liver repopulation by transplanted hepatocytes by deliberately injuring the host cells [97], or "host preconditioning." When irradiated with ionizing radiations like X-rays or gamma rays, donor cells exhibit a higher growth potential over the host's liver cells [98]. Donor hepatocyte numbers can gradually increase in the host from a scattered population, to ~20% of all hepatocytes in 3 weeks, to a complete repopulation of the host liver in 12 weeks. This preparative method can also be applied clinically with stereotactic radiosurgery or 3D conformal radiation therapy prior to cell transplantation [99] thereby facilitating the repopulation. Alternatively, hypoxic preconditioning has been shown [100] to alleviate reperfusion injury, and intermittent hypoxia could cause activation of the anti-apoptotic Bcl-2 pathway in hepatocytes [101]. When combined with other approaches in a multi-pronged strategy, preconditioning may be able to improve engraftment efficiency and proliferation of donor cells in host liver [102]. The comparison of different delivery vehicles has been summarized in Table 1. Under what circumstances the patient would benefit from such an approach should be evaluated relative to overall clinical outcomes, not just relative to duration of donor cell survival.

4.1. Scaffolds

Liver cells, especially hepatocytes, are highly sensitive to their micro-environment [103]. The growth [44], differentiation [104] and health state [105] of hepatocytes are influenced by environmental factors. In initial experiments, cells were injected directly into the liver or the nearby vascular systems. In 1988, liver cells were attached to bio-erodable artificial polymers (polyglactin 910, poly orthoester and polyanhydride) and transplanted into Sprague-Drawley rats, leading to enhanced survival compared with the cell-only control [106]. This early study highlighted the importance of the extracellular environment and showed how it can be engineered with biodegradable polymer-based scaffolds in cell transplantation.

4.1.1. Collagen-based scaffolds

Collagen is a natural ECM polymer that is widely used as a scaffold for hepatocyte transplantation. Hepatocytes attached to collagen-coated micro-carriers [107,108] were transplanted; levels of albumin increased for 4 weeks after intraperitoneal injection into congenic Gunn rats; and the cells were retained in the system for 2 months. Micro-carrier technology continues to advance [109] which may potentiate the application of collagen-based scaffolds.

4.1.2. Galactose-based scaffolds

Galactose modified biomaterials can enhance hepatocyte survival and metabolic functions. Biomaterials for scaffolds have been derived from either natural or synthetic polymers [110]. Rat hepatocytes (65–

100%) could successfully adhere onto poly (acrylamide) gels covalently linked to beta D-galactoside when the glycoside concentration was higher (reached maximal adhesion rate for 20% higher) than the critical concentration of 900 nmol per cm^2 [111]. The physical properties of the galactosylated substratum remain a key factor for improved adhesion since the interactions with cells occur on the scaffold surface. Cells remained as cuboidal three dimensional (3D) cells, avoiding the spreading events onto rigid 2D substratum that are associated with proliferation and with deteriorating hepatocyte functions. We have developed a series of galactosylated membranes to promote hepatocyte attachment and to maintain cell functions in extracorporeal bio-artificial liver assist devices. A 3D hepatocyte monolayer on poly-terephthalate film was developed with the use of Arg-Gly-Asp (RGD) peptides and galactose ligands [112] to enhance adhesion and function synergistically. The hepatocytes formed spheroids and were mechanically tethered onto the substratum. The functional markers from the 3D monolayer system (albumin, urea secretion, EROD activity) were comparable to the levels from hepatocytes in un-tethered 3D spheroid configuration [113]. The 3D monolayer configuration eliminated the high detachment rate and poor mass transport associated with spheroids and the future versions could employ biodegradable biomaterials for cell transplantation.

Galactose-derived Pluronic F68 (F68-Gal) was attached onto polyvinylidene difluoride (PVDF) surfaces via hydrophobic interaction [114]. The modified substratum is stable (remained unchanged for 11 days in hepatocyte culture medium) and allowed a high efficiency (74%) of hepatocyte attachment similar to the collagen-coated PVDF membranes (80%). The albumin synthesis level of F68-Gal coated PVDF membranes was significantly higher throughout the study than the collagen-coated membrane control.

4.1.3. Hydrogels

Fibrin is a widely used injectable scaffold for cell transplantation, owing to its biodegradability and support for cell infiltration and proliferation [115]. Hepatocytes (1.5×10^7 cells) mixed with 100 mg fibrinogen were injected into the peritoneal cavity of athymic mice to maintain the animal's albumin synthesis and glycogen storage functions for at least one week after transplantation [116]. An injectable, bio-degradable, thermo-sensitive hydrogel [117] was developed using a copolymer of poly (organophosphazene) to preserve the hepatocyte spheroids' viability and morphology, allowing maintenance of differentiated structures and functions. When compared to single-cell hepatocyte control, the spheroids maintained higher viability and higher production of albumin or urea for 28 days. Cell death in spheroidal hepatocytes was 10%, while that in single-cell hepatocytes exceeded 50% after 7-day culture. This technology was developed for use in bioreactors *in vitro* but can be further modified for cell transplantation.

4.2. Three-dimensional microenvironment

There is an evolving idea that a three-dimensional environment can support liver cells and simulate the natural environment better than a two dimensional substratum [118]. C3A cells attached to 3D collagen gels were immobilized on polyether sulfone membranes and transplanted intraperitoneally near the liver of SCID/NOD mice. The collagen addition increased the capacity of the membrane so it could support 10 times more cells with improved viability (>95%) than the 2D control. Surprisingly, the albumin secretion, a marker of liver function, was unchanged for 7 days after transplantation, and then improved subsequently [119].

Hepatocytes could be attached onto pre-vascularized 3D polyvinyl alcohol (PVA) matrices (high porosity, 95% and pore size 300–400 μm) and 1×10^7 cells were successfully transplanted via portocaval shunt [120]. Hepatocyte transplantations onto biodegradable polymers could maintain their stability for up to 6 months [121] after which the polymer started to degrade *in vitro*. It showed a consistent increase in hepatocyte repopulation with 3D matrices (from 1 week post-transplantation) despite the decreasing hepatocyte numbers on the matrices within the first week. Although the polymeric material eventually developed bio-compatibility issues and could only achieve sub-optimal initial engraftment, the steady increase in cell number and maintenance of albumin production was observed up to 1 year after transplantation.

4.2.1. Encapsulation

Encapsulating cells can avoid some aspects of immune rejection and can prolong survival. For example, intrasplenic transplantation of 5×10^5 rat hepatocytes encapsulated within 4 cm-long PVDF hollow fibers survived 28 days [122]. Transplantation of 2×10^7 hepatocytes encapsulated in alginate poly-L-lysine microcapsules (500 μm in diameter) in FHF model (male Lewis rats) led to a decrease in mortality and improvement in liver functions (strong albumin expression) [123]. Alginate based encapsulation technologies have been extensively researched as alginate-poly-L-lysine-alginate microcapsules (APA) [124,125] or as alginate-chitosan microcapsules (AC) [126,127]. We have encapsulated hepatocytes in a two-layered polymeric microcapsule [41] that was permeable to small molecules (nutrients, growth factors and metabolites) but was inaccessible to larger molecules like immunoglobulins. 10% terpolymer and 1.5 mg/ml of collagen were used to create the two layers: a thin outer layer (2–3 μm) and a soft gel-like inner layer, which mimics the loose ECM *in vivo*. Improved multi-layered micro-capsules [128] have independently addressed mechanical stability [129], complete cell encapsulation, selective permeability and a favorable micro-environment for enhancing cellular functions. Encapsulation helped to circumvent the immune barrier and sustained the transplanted cells. However, the immune response to encapsulated hepatocytes does exist [130]. *In vitro* and *in vivo* experiments have demonstrated that anti-HepG2 antibody was detectable from day 3 onwards in the supernatant of a co-culture system and the serum of rats that were transplanted with encapsulated HepG2 cells [130], indicating immune rejection occurs to encapsulated cells.

4.3. Vascularization

One major bottleneck affecting the fate of scaffold-based transplanted cells is insufficient vascularization. Some studies [131–133] have shown that blood vessels penetrate into porous scaffolds after a 2 week-process of vascular growth. Empty PVA sponges were implanted into the subcutaneous tissue of athymic nude rats [134]. Fibro-vascular tissue in-growth occurred in 5 days prior to the transplantation of human hepatocytes onto the sponges. Re-organization of the hepatic parenchyma was observed 9 days after the hepatocyte transplantation (36% increase in engraftment was observed within 3 days). Another strategy is co-administration of angiogenic factors to enhance the vascularization into scaffolds. Hepatocytes seeded on pre-vascularized scaffolds (with controlled release of VEGF) were transplanted into the male Lewis rat liver. The enhanced vascularization improved the hepatocyte engraftment (hepatocyte area was 4.6 times higher than in the control rats) [135]. However, one contradictory finding from a similar approach concluded no increase in survival for subcutaneously transplanted hepatocytes in SCID mouse, even after administration of hepatotrophic factors like epidermal growth factor (EGF) and/or HGF in addition to VEGF [136]. They observed a drastic decrease in the hepatocyte survival at 7 and 14 days after treatment, falling to 10–20% of the day 3 values. Different sites of cell transplantation may be a reason for the different findings in these studies. Even with these controversies, the combination of growth factors with biomaterial-based cell delivery has generally led to improvements in the survival of transplanted hepatocytes, either by enhancing the proliferation rate or promoting the vascularization of the scaffold [137].

To create a conducive environment for native liver regeneration (versus merely focusing on extended cell engraftment), we should shift the direction of delivery vehicle development. Be it scaffolds, microenvironments, or controlled release of factors for vascularization, the delivery vehicles optimized for maximal cell engraftment and long-term survival would be different from the ones that maximize short-term direct impact in/near liver to stimulate native liver regeneration. For instance, encapsulation technologies [138] that were developed to avoid host immune system would protect the encapsulated cells with a secure barrier which unfortunately also limits cell-interactions with the host environments. Controlled release of the proteins secreted by the cells on PLG scaffolds can fine-tune the cellular behaviors but it might not be as effective in impacting the native liver regeneration with a large burst release of proteins [139]. Therefore, future scaffolds, microenvironments, and vascularization should permit greater degree of cell–cell interaction and molecular signal access.

5. Sites of cell transplantation

Cells for transplantation have traditionally been delivered to liver through one of the major components of the liver i.e., hepatic portal vein [140,141] or hepatic artery [142]. The hepatic portal vein drains blood from the gastrointestinal tract and spleen into the liver. It is more often used compared to the hepatic artery because multiple vascular accesses are more practical through the vein. The other major route of injecting cells into liver is via spleen, usually the splenic pulp. A comparison of different sites for cell transplantation is summarized in Table 1. The intrasplenic route is commonly used because many of the cells injected into the spleen have been shown to migrate to the liver [143]. Though highly efficient, direct deliveries into liver might pose the risk of occlusion and in certain cases fibrosis, due to portal hypertension and embolism of cells. A hybrid approach was to encapsulate hepatocytes in an isolated spleen as a carrier, and then attach the spleen onto the peritoneal side of the right lobe of the recipient liver using a biodegradable adhesive [144]. The transplanted spleen containing hepatocytes could establish circulation with the host (intact and pinkish suggesting influx of red blood cells). The hepatocytes survived and the albumin secretion started from day 3 and increased with time.

5.1. Spleen

The spleen is a natural location for transplanting cells for treating liver diseases [9,141,145,146] because it has a rich blood supply which is accessible to hepatic portal circulation, leading to the translocation of the transplanted cells to the hepatic sinusoids. In certain cases spleen hepatization can occur. For example, in the study of Strom et al. [14], 40% of the spleen was replaced by transplanted hepatocytes and donor hepatocytes repopulated up to 97% of the host liver, in mice with genetically induced liver disease. One third of the transplanted cells gave rise to replicating hepatocyte foci which showed an average of 12 cell doublings [147]. Direct intrasplenic injection was suggested as a better method to transplant hepatocytes compared to the splenic artery infusion, since the latter led to vascular occlusion with hepatocytes, gastric erosion, and large areas of splenic necrosis [148]. Radioactive labeling of intrasplenically transplanted hepatocytes demonstrated that ~8% reached liver while 20% went to lungs, and less than 1% were retained in the spleen [149]. Although intrasplenic injection is one of the main routes for delivering cells to the liver, there have been reported cases of intrasplenic transplantation leading to local embolus and large numbers of the transplanted cells retained in the spleen pulp [9,150].

5.2. Kidney capsule

The kidney capsule could be used for transplanting hepatocytes [151] but it yielded low survival of the transplanted cells and it had insufficient space for a large number of cells. The survival of the transplanted hepatocytes increased when transplanted under the bilateral kidney capsule spaces [152] in the ECMs obtained from murine Engelbreth–Holm–Swarm tumor cell lines (EHS-ECMs). EHS-ECM contains collagen IV, laminin, small amounts of epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1) that could sustain the hepatocytes for 140 days and form small liver tissues.

5.3. Peritoneum

The peritoneal cavity is another common injection site for cell transplantation therapy in liver diseases because it is anatomically accessible (allowing less invasive surgery), and can accommodate a large number of transplanted cells. Prior to transplantation, cells were attached to extracellular matrix-like collagen in the form of microcarriers [107] or encapsulated in biodegradable polymers [106] or in pre-vascularized PVA sponges [153]. There are many examples of transplanting cells (whether mature hepatocytes or stem cell-derived cells) using this route and it has shown encouraging results in maintaining liver functions and prolonging cell survival [11,154]. The immune response and the delivery between injection site and target site are the main concerns with this route. However, in a recent study, encapsulated porcine hepatocytes could survive and function 1 month post-transplantation in rats without any immune-suppression [155].

5.4. Fat pad

The dorsal interscapular fascia was used as a transplantation site to examine the effect of phenobarbital on hepatocyte proliferation in rats, and the transplanted hepatocytes remained in the transplanted site for at least 21 days [156]. Cells transplanted into both the dorsal and the two anterior lateral fat pads were detectable at 28 days post-transplantation [157]. Although the transplanted cells were not completely functional, they exhibited some functions in the dorsal fat pad microenvironment that are characteristics of hepatocytes (e.g. glutamine synthetase production). Though the peritoneum and fat pads have been used to transplant hepatocytes, these sites are not conducive for long-term cell survival owing to the lack of oxygen and other nutrients. Comparison between the above mentioned routes for transplanting hepatocytes demonstrated that the engraftment and functions were markedly higher in liver or spleen than in the peritoneal cavity or the dorsal fat pads [158].

In general, distant sites may allow transplantation of a greater number of cells, and a larger magnitude has obvious potential benefits for symptomatic relief of metabolic problems. However the benefits to the native liver would be diluted and would be limited to effects mediated by circulating factors. Transplanting liver cells into ectopic sites other than the spleen was associated with lower survival rates of the transplanted cells [158]. Sites that protect the transplanted cells from immune response are advantageous for prolonging the cells' survival, but may have disadvantages similar to distant sites, with dilution of certain benefits to the host environment. Improving the host liver environment to stimulate the regenerative behavior of endogenous hepatocytes might be more easily triggered by transplantation sites in or near the liver. Environmental cues like ECM, nutrients, growth factors [159] also provide the opportunity for essential interactions of the transplanted cells with non-parenchymal cells of the host liver to support the engraftment and prolonged survival of the transplanted cells [160].

6. Limitations of the endogenous regeneration approach

The strategy of promoting regeneration of the native liver is handicapped in cases where the native liver has a permanent defect in

its ability to regenerate, such as mutation of the transcription factor c-Jun [161]. Likewise, regeneration of the native liver is not beneficial in cases where the regenerated liver remain dysfunctional. Inherited metabolic disorders exemplify this category of conditions, and the endogenous regeneration approach we described above would not be useful. For patients with genetic defects in liver functions, transplanted cells must provide sufficient liver functions for life support. The primary objectives of such transplantations are to maximize the survival, engraftment, and functions of the transplanted cells with no concern for endogenous regeneration. Even if the endogenous hepatocytes proliferate to regenerate liver fully, they will still exhibit the same pathological phenotypes.

Cell transplantation for metabolic disorders has been reviewed recently [162] and the following considerations have been suggested: cell dose, variations in the quality of hepatocyte preparations, and rejection or senescence of the transplanted hepatocytes. The most common clinical strategy for cell transplantation to treat inborn metabolic deficits of the liver is a series of multiple transplantations of cryopreserved hepatocytes to be performed for each patient [43]. Cryopreserved hepatocytes have reduced viability (51% to 94%) [43] and/or functions (75%) [11] compared with the freshly isolated cells. Greater longevity of transplanted cells and reduced frequency of transplantation are highly desirable, and permanent engraftment of some metabolically competent cells would be ideal.

Hepatocyte transplantation has been investigated in multiple studies for treating urea cycle disorder [43,163–165]. For example, intraportal transplantation of 4×10^9 cryopreserved allogeneic hepatocytes was used to treat a male infant with urea cycle disorder, once or twice daily when transplantable cells were available. Plasma ammonia and glutamine remained within normal ranges during days 21 to 31 after birth. However, hyperammonemia recurred on day 31 and metabolic stability was reestablished only after liver transplantation when the infant was 6 months old [43]. Transplantation of 3.5–5.6×10^9 cryopreserved hepatocytes has also been used to stabilize patients' metabolic conditions while waiting for liver transplantation [163,165].

Highly significant clinical benefits have been reported after hepatocyte transplantation for patients with Crigler–Najjar syndrome [42], Refsum disease [166], factor VII deficiency [167], and glycogen storage disease [168]. A patient with Crigler–Najjar syndrome received a transplantation of hepatocytes, resulting in multiple markers of disease improvement including a decline in total serum bilirubin of 50%, and decreasing phototherapy from 12 h to 6 h per day, which improved the patient's quality of life and provided a post-transplant survival of more than 18 months [169]. After hepatocyte transplantation, a child with infantile Refsum's disease had a partial correction in the metabolic abnormality and persistent evidence of peroxysomal function up to 18 months [166]. Factor VII deficiency is a rare autosomal recessive disorder; three related boys underwent hepatocyte transplantation resulting in a decrease in the requirement for recombinant factor VII at 6 months to 20% of the pre-transplant levels [170]. In glycogen storage disease type Ia, hepatocyte transplantation resulted in improved glucose control on a normal diet for two of the patients, and one of the patients showed normal glucose 6 phosphatase activity for 7 months [168]. Multiple studies agree that transplantations of hepatocytes can serve as a bridge for patients with various metabolic disorders of the liver while they wait for liver transplantation. An alternative approach to treating metabolic disorders is gene therapy for restoring missing functions, such as with *ex vivo* genetically modified hepatocytes for autologous cell transplantation [171].

7. Outlook

Cell transplantation has provided improved liver functions, alleviation of symptoms, and other measures of improved health to a spectrum of patients with acute liver failure, chronic liver failure, and inherited metabolic disorders [10,14,43,167,168,172]. The procedure is considerably less invasive than organ transplantation and cryopreserved cells are available immediately for treatment of acute liver failure. Hepatocyte transplantation may be able to trigger aspects of regenerative behavior in the host liver which are not activated by other types of cell transplantation. This could explain why the unique advantages of the more primitive cell types have not caused significant improvement in clinical outcomes relative to mature hepatocytes. If using large doses of highly functional hepatocytes for switching environmental cues and stimulating endogenous hepatocyte regeneration is indeed an effective approach, then additional degrees of freedom will be available for future choices of cell sources, delivery vehicles and target sites. As examples, immune rejection against xenogenic or highly differentiated cells might not be as great a concern; more biocompatible but faster degrading scaffolds or hydrogels might become suitable; and hepatic routes that deliver directly to liver environments might become the preferred sites in cell transplantations. Future research with stem or progenitor cells, and/or tissue engineering methods may yield improved ways to maintain hepatocyte-like functions (synthesis of factors, metabolic capacity, etc.) in liver diseases, as well as ways to enhance proliferation, engraftment, survival and other desirable features for cell transplantation therapy. We envision future efforts that combine the proliferative and integrative capabilities of stem or progenitor cells with the specialized functions and environment-changing impact of mature hepatocytes (natural or *in vitro* differentiated) or drug/gene therapies in the treatment of liver diseases in the coming years.

Stem or progenitor cells, in particular iPS cells, are an exciting prospect for future cell transplantations and may prove a sustainable alternative source of cells, provided that tumorigenicity concerns are addressed and high levels of hepatocyte-like functions can be induced. Innovations aimed at enriching the quality of current cell sources and improving hepatocyte functions, survival and delivery will certainly help improve clinical outcomes. The distinction we have emphasized here between regenerative- and engraftment-oriented approaches is forced by the current limitations of available cells, vehicles, and target sites. However, the future of cell transplantation therapies may eventually surpass these concerns by combining the best features of all approaches.

Acknowledgements

This work is supported in part by the Institute of Bioengineering and Nanotechnology (IBN); extramural grants (R185-001-045-305) from BMRC, A*STAR; (R-185-000-135-112) from ARC; (R-185-000-099-213) from NMRC; (R-185-000-182-592) from Janssen Cilag; (C382-641-001-091) from SMA, SMART and Centre for Mechanobiology to HYU; by a Lee Kuan Yew postdoctoral fellowship (R-252-000-342-112) and SMA grant (C-382-641-004-091) to LTK.

References

[1] A.A. Demetriou, R.S. Brown Jr., R.W. Busuttil, J. Fair, B.M. McGuire, et al., Prospective, randomized, multicenter, controlled trial of a bioartificial liver in treating acute liver failure, Ann. Surg. 239 (2004) 660–667 discussion 667–670.
[2] M.F. Chen, T.L. Hwang, C.F. Hung, Human liver regeneration after major hepatectomy. A study of liver volume by computed tomography, Ann. Surg. 213 (1991) 227–229.
[3] R.J. Fontana, Acute liver failure including acetaminophen overdose, Med. Clin. North Am. 92 (2008) 761–794 viii.
[4] J.A. Fallowfield, T.J. Kendall, J.P. Iredale, Reversal of fibrosis: no longer a pipe dream? Clin. Liver Dis. 10 (2006) 481–497 viii.
[5] J.F. Gallegos-Orozco, H.E. Vargas, Liver transplantation: from Child to MELD, Med. Clin. North Am. 93 (2009) 931–950 ix.
[6] J. Phua, K.H. Lee, Liver support devices, Curr. Opin. Crit. Care 14 (2008) 208–215.
[7] S.J. Karp, Clinical implications of advances in the basic science of liver repair and regeneration, Am. J. Transplant. 9 (2009) 1973–1980.

[8] A. Sgroi, V. Serre-Beinier, P. Morel, L. Buhler, What clinical alternatives to whole liver transplantation?. Current status of artificial devices and hepatocyte transplantation, Transplantation 87 (2009) 457–466.
[9] M. Mito, H. Ebata, M. Kusano, T. Onishi, T. Saito, et al., Morphology and function of isolated hepatocytes transplanted into rat spleen, Transplantation 28 (1979) 499–505.
[10] B.M. Bilir, D. Guinette, F. Karrer, D.A. Kumpe, J. Krysl, et al., Hepatocyte transplantation in acute liver failure, Liver Transpl. 6 (2000) 32–40.
[11] C.M. Habibullah, I.H. Syed, A. Qamar, Z. Taher-Uz, Human fetal hepatocyte transplantation in patients with fulminant hepatic failure, Transplantation 58 (1994) 951–952.
[12] M. Mito, M. Kusano, Y. Kawaura, Hepatocyte transplantation in man, Transplant. Proc. 24 (1992) 3052–3053.
[13] A. Schneider, M. Attaran, P.N. Meier, C. Strassburg, M.P. Manns, et al., Hepatocyte transplantation in an acute liver failure due to mushroom poisoning, Transplantation 82 (2006) 1115–1116.
[14] S.C. Strom, R.A. Fisher, M.T. Thompson, A.J. Sanyal, P.E. Cole, et al., Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure, Transplantation 63 (1997) 559–569.
[15] D. Haridass, Q. Yuan, P.D. Becker, T. Cantz, M. Iken, et al., Repopulation efficiencies of adult hepatocytes, fetal liver progenitor cells, and embryonic stem cell-derived hepatic cells in albumin-promoter-enhancer urokinase-type plasminogen activator mice, Am. J. Pathol. 175 (2009) 1483–1492.
[16] J.M. Wilson, Round two for liver gene therapy, Nat. Genet. 12 (1996) 232–233.
[17] C. Christophi, N. Harun, T. Fifis, Liver regeneration and tumor stimulation — a review of cytokine and angiogenic factors, J. Gastrointest. Surg. 12 (2008) 966–980.
[18] N. Nagasue, H. Yukaya, Y. Ogawa, H. Kohno, T. Nakamura, Human liver regeneration after major hepatic resection. A study of normal liver and livers with chronic hepatitis and cirrhosis, Ann. Surg. 206 (1987) 30–39.
[19] J.M. Schoen Smith, W.W. Lautt, The role of prostaglandins in triggering the liver regeneration cascade, Nitric Oxide 13 (2005) 111–117.
[20] W.E. Russell, R.J. Coffey, A.J. Ouellette, H.L. Moses, Type beta transforming growth factor reversibly inhibits the early proliferative response to partial hepatectomy in the rat, Proc. Natl Acad. Sci. USA 85 (1988) 5126–5130.
[21] A.M. Gressner, B. Lahme, H.G. Mannherz, B. Polzar, TGF-beta-mediated hepatocellular apoptosis by rat and human hepatoma cells and primary rat hepatocytes, J. Hepatol. 26 (1997) 1079–1092.
[22] R. Taub, Liver regeneration: from myth to mechanism, Nat. Rev. Mol. Cell Biol. 5 (2004) 836–847.
[23] S. Werner, R. Grose, Regulation of wound healing by growth factors and cytokines, Physiol. Rev. 83 (2003) 835–870.
[24] R. Bataller, D.A. Brenner, Liver fibrosis, J. Clin. Invest. 115 (2005) 209–218.
[25] W. Jiang, S. Hiscox, K. Matsumoto, T. Nakamura, Hepatocyte growth factor/scatter factor, its molecular, cellular and clinical implications in cancer, Crit. Rev. Oncol. Hematol. 29 (1999) 209–248.
[26] H.S. Lee, A.M. Huang, G.T. Huang, P.M. Yang, P.J. Chen, et al., Hepatocyte growth factor stimulates the growth and activates mitogen-activated protein kinase in human hepatoma cells, J. Biomed. Sci. 5 (1998) 180–184.
[27] E. Granot, P. Boros, C.M. Miller, Differential effect of hepatocyte growth factor and tumor growth factor-beta on early release of vascular endothelial growth factor from HepG2 cells: possible implications in post-transplant liver regeneration, Transplant. Proc. 33 (2001) 2926–2928.
[28] W.T. Monacci, M.J. Merrill, E.H. Oldfield, Expression of vascular permeability factor/vascular endothelial growth factor in normal rat tissues, Am. J. Physiol. 264 (1993) C995–C1002.
[29] Y. Saitou, K. Shiraki, Y. Yamaguchi, T. Nakano, S. Mizuno, et al., Serum vascular endothelial growth factor-receptor 1 during liver regeneration, J. Hepatol. 41 (2004) 170–171.
[30] B. Carpenter, Y. Lin, S. Stoll, R.L. Raffai, R. McCuskey, et al., VEGF is crucial for the hepatic vascular development required for lipoprotein uptake, Development 132 (2005) 3293–3303.
[31] M. Uno, S. Kurita, H. Misu, H. Ando, T. Ota, et al., Tranilast, an antifibrogenic agent, ameliorates a dietary rat model of nonalcoholic steatohepatitis, Hepatology 48 (2008) 109–118.
[32] S.S. Yang, C.C. Huang, J.R. Chen, C.L. Chiu, M.J. Shieh, et al., Effects of ethanol on antioxidant capacity in isolated rat hepatocytes, World J. Gastroenterol. 11 (2005) 7272–7276.
[33] L. Makowka, R.E. Falk, L.E. Rotstein, J.A. Falk, N. Nossal, et al., Cellular transplantation in the treatment of experimental hepatic failure, Science 210 (1980) 901–903.
[34] L. Makowka, L.E. Rotstein, R.E. Falk, J.A. Falk, R. Zuk, et al., Allogeneic and xenogeneic hepatocyte transplantation, Transplant. Proc. 13 (1981) 855–859.
[35] L. Makowka, L.E. Rotstein, R.E. Falk, J.A. Falk, R. Zuk, et al., Studies into the mechanism of reversal of experimental acute hepatic failure by hepatocyte transplantation. 1, Can. J. Surg. 24 (1981) 39–44.
[36] A. Leask, TGFbeta, cardiac fibroblasts, and the fibrotic response, Cardiovasc. Res. 74 (2007) 207–212.
[37] N. Gupta, X. Su, B. Popov, J.W. Lee, V. Serikov, et al., Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice, J. Immunol. 179 (2007) 1855–1863.
[38] A. Desmouliere, A. Geinoz, F. Gabbiani, G. Gabbiani, Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts, J. Cell Biol. 122 (1993) 103–111.
[39] D. van Poll, B. Parekkadan, C.H. Cho, F. Berthiaume, Y. Nahmias, et al., Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo, Hepatology 47 (2008) 1634–1643.
[40] H.C. Fiegel, U. Kneser, D. Kluth, R. Metzger, H. Till, et al., Development of hepatic tissue engineering, Pediatr. Surg. Int. 25 (2009) 667–673.
[41] S.M. Chia, K.W. Leong, J. Li, X. Xu, K. Zeng, et al., Hepatocyte encapsulation for enhanced cellular functions, Tissue Eng. 6 (2000) 481–495.
[42] I.J. Fox, J.R. Chowdhury, S.S. Kaufman, T.C. Goertzen, N.R. Chowdhury, et al., Treatment of the Crigler–Najjar syndrome type I with hepatocyte transplantation, N. Engl. J. Med. 338 (1998) 1422–1426.
[43] S.P. Horslen, T.C. McCowan, T.C. Goertzen, P.I. Warkentin, H.B. Cai, et al., Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder, Pediatrics 111 (2003) 1262–1267.
[44] A. Martinez-Hernandez, P.S. Amenta, The hepatic extracellular matrix. II. Ontogenesis, regeneration and cirrhosis, Virchows Arch. A Pathol. Anat. Histopathol. 423 (1993) 77–84.
[45] L. Venkatraman, H. Yu, S.S. Bhowmick, C.F. Dewey Jr., L. Tucker-Kellogg, The Steady States and Dynamics of Urokinase-mediated Plasmin Activation, Pac. Symp. Biocomput. (2010) 190–200.
[46] A. Sgroi, V. Serre-Beinier, P. Morel, L. Buhler, What clinical alternatives to whole liver transplantation: current status of artificial devices and hepatocyte transplantation, Transplantation 87 (2009) 457–466.
[47] C. Terry, R.D. Hughes, An optimised method for cryopreservation of human hepatocytes, Methods Mol. Biol. 481 (2009) 25–34.
[48] D.C. Hay, J. Fletcher, C. Payne, J.D. Terrace, R.C. Gallagher, et al., Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 12301–12306.
[49] N.M. Meindl-Beinker, S. Dooley, Transforming growth factor-beta and hepatocyte transdifferentiation in liver fibrogenesis, J. Gastroenterol. Hepatol. 23 (Suppl 1) (2008) S122–S127.
[50] H. Watanabe, M. Hata, N. Terada, H. Ueda, N. Yamada, et al., Transdifferentiation into biliary ductular cells of hepatocytes transplanted into the spleen, Pathology 40 (2008) 272–276.
[51] M. Zeisberg, A.A. Shah, R. Kalluri, Bone morphogenic protein-7 induces mesenchymal to epithelial transition in adult renal fibroblasts and facilitates regeneration of injured kidney, J. Biol. Chem. 280 (2005) 8094–8100.
[52] M. Zeisberg, C. Yang, M. Martino, M.B. Duncan, F. Rieder, et al., Fibroblasts derive from hepatocytes in liver fibrosis via epithelial to mesenchymal transition, J. Biol. Chem. 282 (2007) 23337–23347.
[53] S.C. Lehec, R.D. Hughes, R.R. Mitry, M.A. Graver, A. Verma, et al., Experience of microbiological screening of human hepatocytes for clinical transplantation, Cell Transplant. (2009).
[54] A.T. Blei, Selection for acute liver failure: have we got it right? Liver Transpl. 11 (2005) S30–S34.
[55] S.M. Riordan, R. Williams, Treatment of hepatic encephalopathy, N. Engl. J. Med. 337 (1997) 473–479.
[56] A.A. Khan, A. Habeeb, N. Parveen, B. Naseem, R.P. Babu, et al., Peritoneal transplantation of human fetal hepatocytes for the treatment of acute fatty liver of pregnancy: a case report, Trop. Gastroenterol. 25 (2004) 141–143.
[57] N. Kobayashi, H. Noguchi, T. Watanabe, T. Matsumura, T. Totsugawa, et al., Establishment of a tightly regulated human cell line for the development of hepatocyte transplantation, Hum. Cell 13 (2000) 7–13.
[58] S.C. Strom, R.A. Fisher, W.S. Rubinstein, J.A. Barranger, R.B. Towbin, et al., Transplantation of human hepatocytes, Transplant. Proc. 29 (1997) 2103–2106.
[59] D.M. Dalgetty, C.N. Medine, J.P. Iredale, D.C. Hay, Progress and future challenges in stem cell-derived liver technologies, Am. J. Physiol. Gastrointest. Liver Physiol. 297 (2009) G241–G248.
[60] Q. Zhao, H. Ren, D. Zhu, Z. Han, Stem/progenitor cells in liver injury repair and regeneration, Biol. Cell 101 (2009) 557–571.
[61] A. Weber, M.T. Groyer-Picard, D. Franco, I. Dagher, Hepatocyte transplantation in animal models, Liver Transpl. 15 (2009) 7–14.
[62] L. Pi, S.H. Oh, T. Shupe, B.E. Petersen, Role of connective tissue growth factor in oval cell response during liver regeneration after 2-AAF/PHx in rats, Gastroenterology 128 (2005) 2077–2088.
[63] J.M. Lemire, N. Shiojiri, N. Fausto, Oval cell proliferation and the origin of small hepatocytes in liver injury induced by D-galactosamine, Am. J. Pathol. 139 (1991) 535–552.
[64] X. Wang, M. Foster, M. Al-Dhalimy, E. Lagasse, M. Finegold, et al., The origin and liver repopulating capacity of murine oval cells, Proc. Natl. Acad. Sci. U. S. A. 100 (Suppl 1) (2003) 11881–11888.
[65] M.I. Yovchev, P.N. Grozdanov, H. Zhou, H. Racherla, C. Guha, et al., Identification of adult hepatic progenitor cells capable of repopulating injured rat liver, Hepatology 47 (2008) 636–647.
[66] C.X. Wu, Q. Zou, Z.Y. Zhu, Y.T. Gao, Y.J. Wang, Intrahepatic transplantation of hepatic oval cells for fulminant hepatic failure in rats, World J. Gastroenterol. 15 (2009) 1506–1511.
[67] J. Cai, Y. Zhao, Y. Liu, F. Ye, Z. Song, et al., Directed differentiation of human embryonic stem cells into functional hepatic cells, Hepatology 45 (2007) 1229–1239.
[68] T. Teratani, H. Yamamoto, K. Aoyagi, H. Sasaki, A. Asari, et al., Direct hepatic fate specification from mouse embryonic stem cells, Hepatology 41 (2005) 836–846.
[69] Y. Kumashiro, K. Asahina, R. Ozeki, K. Shimizu-Saito, Y. Tanaka, et al., Enrichment of hepatocytes differentiated from mouse embryonic stem cells as a transplantable source, Transplantation 79 (2005) 550–557.

[70] R. Zemel, L. Bachmatov, D. Ad-El, R. Tur-Kaspa, Abstracts of the 43 rd Annual Meeting of the European Association for the Study of the Liver, April 23–27, 2008, Milan, Italy, J. Hepatol. 48 (Suppl 2) (2008) S200–S201.
[71] A. Banas, T. Teratani, Y. Yamamoto, M. Tokuhara, F. Takeshita, et al., Rapid hepatic fate specification of adipose-derived stem cells and their therapeutic potential for liver failure, J. Gastroenterol. Hepatol. 24 (2009) 70–77.
[72] P.C. Tsai, T.W. Fu, Y.M. Chen, T.L. Ko, T.H. Chen, et al., The therapeutic potential of human umbilical mesenchymal stem cells from Wharton's jelly in the treatment of rat liver fibrosis, Liver Transpl. 15 (2009) 484–495.
[73] H. Malhi, A.N. Irani, S. Gagandeep, S. Gupta, Isolation of human progenitor liver epithelial cells with extensive replication capacity and differentiation into mature hepatocytes, J. Cell Sci. 115 (2002) 2679–2688.
[74] M. Oertel, A. Menthena, Y.Q. Chen, B. Teisner, C.H. Jensen, et al., Purification of fetal liver stem/progenitor cells containing all the repopulation potential for normal adult rat liver, Gastroenterology 134 (2008) 823–832.
[75] J.S. Sandhu, P.M. Petkov, M.D. Dabeva, D.A. Shafritz, Stem cell properties and repopulation of the rat liver by fetal liver epithelial progenitor cells, Am. J. Pathol. 159 (2001) 1323–1334.
[76] Y. Yokoyama, M. Nagino, Y. Nimura, Mechanisms of hepatic regeneration following portal vein embolization and partial hepatectomy: a review, World J. Surg. 31 (2007) 367–374.
[77] F. Anjos-Afonso, E.K. Siapati, D. Bonnet, In vivo contribution of murine mesenchymal stem cells into multiple cell-types under minimal damage conditions, J. Cell Sci. 117 (2004) 5655–5664.
[78] E. Lagasse, H. Connors, M. Al-Dhalimy, M. Reitsma, M. Dohse, et al., Purified hematopoietic stem cells can differentiate into hepatocytes in vivo, Nat. Med. 6 (2000) 1229–1234.
[79] B. Parekkadan, D. van Poll, K. Suganuma, E.A. Carter, F. Berthiaume, et al., Mesenchymal stem cell-derived molecules reverse fulminant hepatic failure, PLoS ONE 2 (2007) e941.
[80] M.R. Alison, R. Poulsom, R. Jeffery, A.P. Dhillon, A. Quaglia, et al., Hepatocytes from non-hepatic adult stem cells, Nature 406 (2000) 257.
[81] M. Korbling, R.L. Katz, A. Khanna, A.C. Ruifrok, G. Rondon, et al., Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells, N. Engl. J. Med. 346 (2002) 738–746.
[82] N.D. Theise, M. Nimmakayalu, R. Gardner, P.B. Illei, G. Morgan, et al., Liver from bone marrow in humans, Hepatology 32 (2000) 11–16.
[83] D. Xu, Z. Alipio, L.M. Fink, D.M. Adcock, J. Yang, et al., Phenotypic correction of murine hemophilia A using an iPS cell-based therapy, Proc. Natl. Acad. Sci. U S A 106 (2009) 808–813.
[84] S.S. Chen, W. Fitzgerald, J. Zimmerberg, H.K. Kleinman, L. Margolis, Cell–cell and cell–extracellular matrix interactions regulate embryonic stem cell differentiation, Stem Cells 25 (2007) 553–561.
[85] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, Cell 126 (2006) 677–689.
[86] M. Zeisberg, K. Kramer, N. Sindhi, P. Sarkar, M. Upton, et al., De-differentiation of primary human hepatocytes depends on the composition of specialized liver basement membrane, Mol. Cell. Biochem. 283 (2006) 181–189.
[87] S. Benoist, R. Sarkis, V. Barbu, J. Honiger, M. Baudrimont, et al., Survival and functions of encapsulated porcine hepatocytes after allotransplantation or xenotransplantation without immunosuppression, Surgery 129 (2001) 606–616.
[88] H. Nagata, M. Ito, J. Cai, A.S. Edge, J.L. Platt, et al., Treatment of cirrhosis and liver failure in rats by hepatocyte xenotransplantation, Gastroenterology 124 (2003) 422–431.
[89] H. Nagata, R. Nishitai, C. Shirota, J.L. Zhang, C.A. Koch, et al., Prolonged survival of porcine hepatocytes in cynomolgus monkeys, Gastroenterology 132 (2007) 321–329.
[90] M.F. Levy, J. Crippin, S. Sutton, G. Netto, J. McCormack, et al., Liver allotransplantation after extracorporeal hepatic support with transgenic (hCD55/hCD59) porcine livers: clinical results and lack of pig-to-human transmission of the porcine endogenous retrovirus, Transplantation 69 (2000) 272–280.
[91] N. Kobayashi, T. Fujiwara, K.A. Westerman, Y. Inoue, M. Sakaguchi, et al., Prevention of acute liver failure in rats with reversibly immortalized human hepatocytes, Science 287 (2000) 1258–1262.
[92] I.J. Fox, N.R. Chowdhury, S. Gupta, R. Kondapalli, M.L. Schilsky, et al., Conditional immortalization of Gunn rat hepatocytes: an ex vivo model for evaluating methods for bilirubin-UDP-glucuronosyltransferase gene transfer, Hepatology 21 (1995) 837–846.
[93] M. Takahashi, N.J. Deb, Y. Kawashita, S.W. Lee, J. Furgueil, et al., A novel strategy for in vivo expansion of transplanted hepatocytes using preparative hepatic irradiation and FasL-induced hepatocellular apoptosis, Gene Ther. 10 (2003) 304–313.
[94] J. Cai, M. Ito, H. Nagata, K.A. Westerman, D. Lafleur, et al., Treatment of liver failure in rats with end-stage cirrhosis by transplantation of immortalized hepatocytes, Hepatology 36 (2002) 386–394.
[95] K. Kienle, M. Rentsch, T. Muller, N. Engelhard, M. Vogel, et al., Expression of BCL-2 in liver grafts after adenoviral transfer improves survival following prolonged ischemia and reperfusion in rat liver transplantation, Transplant. Proc. 37 (2005) 439–441.
[96] J. Wang, W. Li, J. Min, Q. Ou, J. Chen, et al., Intrasplenic transplantation of allogeneic hepatocytes modified by BCL-2 gene protects rats from acute liver failure, Transplant. Proc. 36 (2004) 2924–2926.
[97] C. Guha, N.J. Deb, B.S. Sappal, S.S. Ghosh, N. Roy-Chowdhury, et al., Amplification of engrafted hepatocytes by preparative manipulation of the host liver, Artif. Organs 25 (2001) 522–528.
[98] C. Guha, B. Parashar, N.J. Deb, A. Sharma, G.R. Gorla, et al., Liver irradiation: a potential preparative regimen for hepatocyte transplantation, Int. J. Radiat. Oncol. Biol. Phys. 49 (2001) 451–457.
[99] H. Zhou, K. Yamanouchi, L. Liu, J. Jiang, S. Mohan, et al., A new paradigm for tissue regeneration: preparative irradiation for cell-based therapies as an alternative to organ transplantation, Int. J. Radiat. Oncol. Biol. Phys. 66 (2006) S98–S99.
[100] A. Schurr, K.H. Reid, M.T. Tseng, C. West, B.M. Rigor, Adaptation of adult brain tissue to anoxia and hypoxia in vitro, Brain Res. 374 (1986) 244.
[101] C. Jin, P.J. Zhang, X.M. Wu, B. Zhou, Y. Li, et al., Impact of hypoxic preconditioning on apoptosis and its possible mechanism in orthotopic liver autotransplantation in rats, Hepatobiliary Pancreat. Dis. Int. 8 (2009) 40–45.
[102] Y.-M. Wu, S. Gupta, Hepatic Preconditioning for Transplanted Cell Engraftment and Proliferation, in: R.D.H. Anil Dhawan (Ed.), Hepatocyte Transplantation, Humana Press, 2009, pp. 1–10.
[103] M.W. Davis, J.P. Vacanti, Toward development of an implantable tissue engineered liver, Biomaterials 17 (1996) 365–372.
[104] D. Mooney, L. Hansen, J. Vacanti, R. Langer, S. Farmer, et al., Switching from differentiation to growth in hepatocytes: control by extracellular matrix, J Cell Physiol 151 (1992) 497–505.
[105] D.M. Bissell, M.O. Choun, The role of extracellular matrix in normal liver, Scand. J. Gastroenterol. Suppl. 151 (1988) 1–7.
[106] J.P. Vacanti, M.A. Morse, W.M. Saltzman, A.J. Domb, A. Perez-Atayde, et al., Selective cell transplantation using bioabsorbable artificial polymers as matrices, J. Pediatr. Surg. 23 (1988) 3–9.
[107] A.A. Demetriou, S.M. Levenson, P.M. Novikoff, A.B. Novikoff, N.R. Chowdhury, et al., Survival, organization, and function of microcarrier-attached hepatocytes transplanted in rats, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 7475–7479.
[108] A.A. Demetriou, J.F. Whiting, D. Feldman, S.M. Levenson, N.R. Chowdhury, et al., Replacement of liver function in rats by transplantation of microcarrier-attached hepatocytes, Science 233 (1986) 1190–1192.
[109] B.A. Justice, N.A. Badr, R.A. Felder, 3D cell culture opens new dimensions in cell-based assays, Drug Discov. Today 14 (2009) 102–107.
[110] C.S. Cho, S.J. Seo, I.K. Park, S.H. Kim, T.H. Kim, et al., Galactose-carrying polymers as extracellular matrices for liver tissue engineering, Biomaterials 27 (2006) 576–585.
[111] P.H. Weigel, E. Schmell, Y.C. Lee, S. Roseman, Specific adhesion of rat hepatocytes to beta-galactosides linked to polyacrylamide gels, J. Biol. Chem. 253 (1978) 330–333.
[112] Y. Du, S.-m. Chia, R. Han, S. Chang, H. Tang, et al., 3D hepatocyte monolayer on hybrid RGD/galactose substratum, Biomaterials 27 (2006) 5669–5680.
[113] Y. Du, R. Han, S. Ng, J. Ni, W. Sun, et al., Identification and characterization of a novel prespheroid 3-dimensional hepatocyte monolayer on galactosylated substratum, Tissue Eng. 13 (2007) 1455–1468.
[114] H.-F. Lu, W.S. Lim, J. Wang, Z.-Q. Tang, P.-C. Zhang, et al., Galactosylated PVDF membrane promotes hepatocyte attachment and functional maintenance, Biomaterials 24 (2003) 4893–4903.
[115] P. Lei, R.M. Padmashali, S.T. Andreadis, Cell-controlled and spatially arrayed gene delivery from fibrin hydrogels, Biomaterials 30 (2009) 3790–3799.
[116] S.J. Gwak, D. Choi, S.S. Paik, E.Y. Lee, K.S. Lees, et al., Stable hepatocyte transplantation using fibrin matrix, Biotechnol. Lett. 26 (2004) 505–508.
[117] K.H. Park, K. Na, S.W. Kim, S.Y. Jung, H.M. Chung, Phenotype of hepatocyte spheroids behavior within thermo-sensitive poly(NiPAAm-co-PEG-g-GRGDS) hydrogel as a cell delivery vehicle, Biotechnol. Lett. 27 (2005) 1081–1086.
[118] A. Abbott, Cell culture: biology's new dimension, Nature 424 (2003) 870–872.
[119] A. Kinasiewicz, K. Dudzinski, A. Chwojnowski, A. Werynski, J. Kawiak, Three-dimensional culture of hepatocytes on spongy polyethersulfone membrane developed for cell transplantation, Transplant. Proc. 39 (2007) 2914–2916.
[120] U. Kneser, P.M. Kaufmann, H.C. Fiegel, J.M. Pollok, D. Kluth, et al., Heterotopic hepatocyte transplantation utilizing pancreatic islet cotransplantation for hepatotrophic stimulation: morphologic and morphometric evaluation, Pediatr. Surg. Int. 15 (1999) 168–174.
[121] D.J. Mooney, K. Sano, P.M. Kaufmann, K. Majahod, B. Schloo, et al., Long-term engraftment of hepatocytes transplanted on biodegradable polymer sponges, J. Biomed. Mater. Res. 37 (1997) 413–420.
[122] T. Aoki, Y. Umehara, C. Ferraresso, N. Sugiyama, Y. Middleton, et al., Intrasplenic transplantation of encapsulated cells: a novel approach to cell therapy, Cell Transplant. 11 (2002) 553–561.
[123] T. Aoki, Z. Jin, N. Nishino, H. Kato, Y. Shimizu, et al., Intrasplenic transplantation of encapsulated hepatocytes decreases mortality and improves liver functions in fulminant hepatic failure from 90% partial hepatectomy in rats, Transplantation 79 (2005) 783–790.
[124] O. Gaserod, A. Sannes, G. Skjak-Braek, Microcapsules of alginate-chitosan. II. A study of capsule stability and permeability, Biomaterials 20 (1999) 773–783.
[125] A.M. Rokstad, S. Holtan, B. Strand, B. Steinkjer, L. Ryan, et al., Microencapsulation of cells producing therapeutic proteins: optimizing cell growth and secretion, Cell Transplant. 11 (2002) 313–324.
[126] T. Haque, H. Chen, W. Ouyang, C. Martoni, B. Lawuyi, et al., Investigation of a new microcapsule membrane combining alginate, chitosan, polyethylene glycol and poly-L-lysine for cell transplantation applications, Int. J. Artif. Organs 28 (2005) 631–637.
[127] T. Haque, H. Chen, W. Ouyang, C. Martoni, B. Lawuyi, et al., In vitro study of alginate-chitosan microcapsules: an alternative to liver cell transplants for the treatment of liver failure, Biotechnol. Lett. 27 (2005) 317–322.
[128] S.M. Chia, A.C. Wan, C.H. Quek, H.Q. Mao, X. Xu, et al., Multi-layered microcapsules for cell encapsulation, Biomaterials 23 (2002) 849–856.

[129] C. Yin, S. Mien Chia, C. Hoon Quek, H. Yu, R.-X. Zhuo, et al., Microcapsules with improved mechanical stability for hepatocyte culture, Biomaterials 24 (2003) 1771–1780.
[130] L. Wen, P. Grude, F. Conti, J. Honiger, J. Capeau, et al., Suppression of humoral immunization against encapsulated xenogeneic hepatocytes and prolongation of their function by 2-week cyclosporine treatment in the rat, Surgery 127 (2000) 301–308.
[131] A.G. Mikos, G. Sarakinos, M.D. Lyman, D.E. Ingber, J.P. Vacanti, et al., Prevascularization of porous biodegradable polymers, Biotechnol. Bioeng. 42 (1993) 716–723.
[132] D.J. Mooney, P.M. Kaufmann, K. Sano, K.M. McNamara, J.P. Vacanti, et al., Transplantation of hepatocytes using porous, biodegradable sponges, Transplant. Proc. 26 (1994) 3425–3426.
[133] W.L. Murphy, M.C. Peters, D.H. Kohn, D.J. Mooney, Sustained release of vascular endothelial growth factor from mineralized poly(lactide-co-glycolide) scaffolds for tissue engineering, Biomaterials 21 (2000) 2521–2527.
[134] M. Fontaine, B. Schloo, R. Jenkins, S. Uyama, L. Hansen, et al., Human hepatocyte isolation and transplantation into an athymic rat, using prevascularized cell polymer constructs, J. Pediatr. Surg. 30 (1995) 56–60.
[135] A. Kedem, A. Perets, I. Gamlieli-Bonshtein, M. Dvir-Ginzberg, S. Mizrahi, et al., Vascular endothelial growth factor-releasing scaffolds enhance vascularization and engraftment of hepatocytes transplanted on liver lobes, Tissue Eng. 11 (2005) 715–722.
[136] M.K. Smith, K.W. Riddle, D.J. Mooney, Delivery of hepatotrophic factors fails to enhance longer-term survival of subcutaneously transplanted hepatocytes, Tissue Eng. 12 (2006) 235–244.
[137] C. Mayer, H.J. Gruber, E.M. Landl, S. Pailer, H. Scharnagl, et al., Rosuvastatin reduces interleukin-6-induced expression of C-reactive protein in human hepatocytes in a STAT3- and C/EBP-dependent fashion, Int. J. Clin. Pharmacol. Ther. 45 (2007) 319–327.
[138] J.M. Karp, R. Langer, Development and therapeutic applications of advanced biomaterials, Curr. Opin. Biotechnol. 18 (2007) 454–459.
[139] A. Murua, A. Portero, G. Orive, R.M. Hernández, M. de Castro, et al., Cell microencapsulation technology: towards clinical application, J. Control. Release 132 (2008) 76–83.
[140] J.R. Gunsalus, D.A. Brady, S.M. Coulter, B.M. Gray, A.S. Edge, Reduction of serum cholesterol in Watanabe rabbits by xenogeneic hepatocellular transplantation, Nat. Med. 3 (1997) 48–53.
[141] K. Overturf, M. Al-Dhalimy, R. Tanguay, M. Brantly, C.N. Ou, et al., Hepatocytes corrected by gene therapy are selected in vivo in a murine model of hereditary tyrosinaemia type I, Nat. Genet. 12 (1996) 266–273.
[142] M.Y. Gordon, N. Levicar, M. Pai, P. Bachellier, I. Dimarakis, et al., Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor, Stem Cells 24 (2006) 1822–1830.
[143] K.P. Ponder, S. Gupta, F. Leland, G. Darlington, M. Finegold, et al., Mouse hepatocytes migrate to liver parenchyma and function indefinitely after intrasplenic transplantation, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 1217–1221.
[144] R. Nishio, M. Nakayama, M. Ikekita, Y. Watanabe, Auxiliary liver organ formation by implantation of spleen-encapsulated hepatocytes, Tissue Eng. 12 (2006) 2565–2572.
[145] M.A. Kay, P. Baley, S. Rothenberg, F. Leland, L. Fleming, et al., Expression of human alpha 1-antitrypsin in dogs after autologous transplantation of retroviral transduced hepatocytes, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 89–93.
[146] N. Kobayashi, M. Ito, J. Nakamura, J. Cai, C. Gao, et al., Hepatocyte transplantation in rats with decompensated cirrhosis, Hepatology 31 (2000) 851–857.
[147] T.C. Weglarz, J.L. Degen, E.P. Sandgren, Hepatocyte transplantation into diseased mouse liver. Kinetics of parenchymal repopulation and identification of the proliferative capacity of tetraploid and octaploid hepatocytes, Am. J. Pathol. 157 (2000) 1963–1974.
[148] H. Nagata, M. Ito, C. Shirota, A. Edge, T.C. McCowan, et al., Route of hepatocyte delivery affects hepatocyte engraftment in the spleen, Transplantation 76 (2003) 732–734.
[149] S. Gupta, C.D. Lee, R.P. Vemuru, K.K. Bhargava, 111Indium labeling of hepatocytes for analysis of short-term biodistribution of transplanted cells, Hepatology 19 (1994) 750–757.
[150] J.P. Vroemen, W.A. Buurman, K.P. Heirwegh, C.J. van der Linden, G. Kootstra, Hepatocyte transplantation for enzyme deficiency disease in congenic rats, Transplantation 42 (1986) 130–135.
[151] K. Ohashi, P.L. Marion, H. Nakai, L. Meuse, J.M. Cullen, et al., Sustained survival of human hepatocytes in mice: a model for in vivo infection with human hepatitis B and hepatitis delta viruses, Nat. Med. 6 (2000) 327–331.
[152] K. Ohashi, M.A. Kay, T. Yokoyama, H. Kuge, H. Kanehiro, et al., Stability and repeat regeneration potential of the engineered liver tissues under the kidney capsule in mice, Cell Transplant. 14 (2005) 621–627.
[153] S. Uyama, P.M. Kaufmann, T. Takeda, J.P. Vacanti, Delivery of whole liver-equivalent hepatocyte mass using polymer devices and hepatotrophic stimulation, Transplantation 55 (1993) 932–935.
[154] I.J. Fox, J.R. Chowdhury, Hepatocyte transplantation, Am. J. Transplant. 4 (Suppl 6) (2004) 7–13.
[155] E. Baldini, R. Cursio, G. De Sousa, A. Margara, J. Honiger, et al., Peritoneal implantation of cryopreserved encapsulated porcine hepatocytes in rats without immunosuppression: viability and function, Transplant. Proc. 40 (2008) 2049–2052.
[156] R.L. Jirtle, G. Michalopoulos, Enhancement of the clonability of adult parenchymal hepatocytes with the liver tumor promoter phenobarbital, Carcinogenesis 7 (1986) 1813–1817.
[157] R. Gebhardt, R. Jirtle, A.F. Moorman, W.H. Lamers, G. Michalopoulos, Induction of glutamine synthetase and transient co-expression with carbamoylphosphate synthetase in hepatocytes transplanted into fat pads of syngeneic hosts, Histochemistry 92 (1989) 337–342.
[158] S. Gupta, R.P. Vemuru, C.D. Lee, P.R. Yerneni, E. Aragona, et al., Hepatocytes exhibit superior transgene expression after transplantation into liver and spleen compared with peritoneal cavity or dorsal fat pad: implications for hepatic gene therapy, Hum. Gene Ther. 5 (1994) 959–967.
[159] S. Gupta, G.R. Gorla, A.N. Irani, Hepatocyte transplantation: emerging insights into mechanisms of liver repopulation and their relevance to potential therapies, J. Hepatol. 30 (1999) 162–170.
[160] S. Gupta, J.R. Chowdhary, Hepatocyte transplantation: back to the future, Hepatology 15 (1992) 156–162.
[161] E. Stepniak, R. Ricci, R. Eferl, G. Sumara, I. Sumara, et al., c-Jun/AP-1 controls liver regeneration by repressing p53/p21 and p38 MAPK activity, Genes Dev. 20 (2006) 2306–2314.
[162] G.M. Enns, M.T. Millan, Cell-based therapies for metabolic liver disease, Mol. Genet. Metab. 95 (2008) 3–10.
[163] J. Meyburg, A.M. Das, F. Hoerster, M. Lindner, H. Kriegbaum, et al., One liver for four children: first clinical series of liver cell transplantation for severe neonatal urea cycle defects, Transplantation 87 (2009) 636–641.
[164] J. Puppi, N. Tan, R.R. Mitry, R.D. Hughes, S. Lehec, et al., Hepatocyte transplantation followed by auxiliary liver transplantation—a novel treatment for ornithine transcarbamylase deficiency, Am. J. Transplant. 8 (2008) 452–457.
[165] X. Stephenne, M. Najimi, F. Smets, R. Reding, J. de Ville de Goyet, et al., Cryopreserved liver cell transplantation controls ornithine transcarbamylase deficient patient while awaiting liver transplantation, Am. J. Transplant. 5 (2005) 2058–2061.
[166] E.M. Sokal, F. Smets, A. Bourgois, L. Van Maldergem, J.P. Buts, et al., Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up, Transplantation 76 (2003) 735–738.
[167] A. Dhawan, R.R. Mitry, R.D. Hughes, S. Lehec, C. Terry, et al., Hepatocyte transplantation for inherited factor VII deficiency, Transplantation 78 (2004) 1812–1814.
[168] M. Muraca, G. Gerunda, D. Neri, M.T. Vilei, A. Granato, et al., Hepatocyte transplantation as a treatment for glycogen storage disease type 1a, Lancet 359 (2002) 317–318.
[169] K.J. Allen, H.E. Soriano, Liver cell transplantation: the road to clinical application, J. Lab. Clin. Med. 138 (2001) 298–312.
[170] A. Dhawan, R.R. Mitry, R.D. Hughes, Hepatocyte transplantation for liver-based metabolic disorders, J. Inherit. Metab. Dis. 29 (2006) 431–435.
[171] T.H. Nguyen, S. Mainot, P. Lainas, M.T. Groyer-Picard, D. Franco, et al., Ex vivo liver-directed gene therapy for the treatment of metabolic diseases: advances in hepatocyte transplantation and retroviral vectors, Curr. Gene Ther. 9 (2009) 136–149.
[172] S.P. Horslen, I.J. Fox, Hepatocyte transplantation, Transplantation 77 (2004) 1481–1486.