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REVIEW PAPER

Liver Tissue Engineering: Recent Advances in the Development of a Bio-artificial Liver

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Abstract Orthotopic liver transplantation is the most common treatment for patients with end-stage liver failure. However, liver transplantation is greatly limited by a donor shortage. Liver tissue engineering may offer a promising strategy to solve this problem by providing transplantable, bioartificial livers. Diverse types of cells, biomaterials, and growth factor delivery systems have been tested for efficient regeneration of liver tissues that possess hepatic functions comparable to native livers. This article reviews recent advances in liver tissue engineering and describes cell sources, biomaterial scaffolds, and growth factor delivery systems that are currently being used to improve the regenerative potential of tissue-engineered livers.

Keywords: liver tissue engineering, biomaterials, scaffolds, hepatocytes, stem cells, growth factor delivery

1. Introduction

The liver is an internal organ that plays an essential role in metabolism [1], detoxification [2], and protein synthesis [3,4]. The high regenerative capacity of injured hepatic tissue [5] allows the liver to recover its original mass and function after a loss of up to 70% of its total mass [6]. Despite this remarkable ability to self-regenerate, whole liver transplants are still the only treatment option for patients with end-stage liver cirrhosis, liver cancer, or chronic liver failure. Unfortunately, this procedure is limited by a donor shortage, immunological complications associated with rejection, and the use of immunosuppressive drugs [6]. Therefore, extracorporeal devices such as artificial livers and cell transplantation techniques have been developed to circumvent the limitations of liver transplantation.

More recently, liver tissue engineering has emerged as a potential technology for the efficient regeneration of liver tissue. Diverse combinations of cell sources and biomaterials are being tested in the context of liver tissue engineering. Growth factor delivery systems that enhance hepatic cell survival and/or angiogenesis are also being tested in order to improve the regenerative potential of these tissue-engineered products. This article reviews recent advances in liver tissue engineering and describes the various cell sources, biomaterials, and growth factor delivery systems currently being used in liver tissue engineering.

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2. Cell Sources for Liver Tissue Engineering

Liver cells are typically selected as the cell source for liver tissue engineering. However, the use of liver cells is limited by the availability of liver tissue for primary isolation, the difficulty in maintaining the hepatic phenotype, and an inability to sufficiently expand the cells [6,7]. Thus, many researchers have sought alternative cell sources for liver tissue engineering. Alternative cells include stem cells or progenitor cells, which have the potential to differentiate into hepatic lineage cells. In this section, we discuss the various cell types that have been used in liver tissue engineering.

2.1. Hepatocytes

Hepatocytes, a principal cell type of the liver, have been used for cell transplantations and in liver tissue engineering. Hepatocytes make up 70 ~ 80% of the liver tissue's cytoplasmic mass and play a major role in metabolic and

enzyme functions [7]. The first clinical trial using autologous hepatocyte transplantation was performed in 1992 [8] and, since then, hepatocyte transplantation has shown therapeutic efficacy for the treatment of acute [9] and chronic liver failure [10,11]. Habibullah *et al.* reported that intraperitoneal infusion of fetal hepatocytes to patients with acute liver failure was able to significantly improve liver function (as measured by a decrease in plasma ammonia and bilirubin levels) and patient survival time without any complications [9]. Transplantation of hepatocytes also improved short-term liver function and alleviated symptoms in patients with chronic liver failure resulting from end-stage liver disease or alcoholic liver cirrhosis [10,11].

However, hepatocytes have practical limitations as a stable cell source for cell therapy or tissue engineering. A donor shortage of liver tissue from which hepatocytes are isolated has become a major limiting factor in using this cell type. In addition, hepatocytes easily lose the hepatic phenotype and functional properties *in vitro* during expansion and following cryopreservation [7,12]. For instance, a previous study demonstrated a 30% reduction in hepatocyte viability after cryopreservation [6,13]. Thus, storage and *in vitro* long-term expansion difficulties do not allow for a sufficient number of functional hepatocytes to be obtained. This reduces the clinical feasibility of using hepatocytes as a source for cell therapy or tissue engineering. The large number of hepatocytes required for transplantation to produce therapeutic effects in humans [14], makes procuring alternative cell sources important for clinical applications.

2.2. Stem cells

Stem cells that can differentiate into hepatic lineage cells are considered a novel cell source of hepatocytes for liver tissue engineering. Although hepatocytes have been widely used as a cell source for cell therapy and liver tissue engineering applications, these cells are not readily available due to the shortage of human liver donors. Thus, researchers have established protocols for generating hepatocytes from a diverse array of stem cells. In fact, hepatocyte-like cells derived from stem cells can regenerate hepatic tissues and restore hepatic function in animal models with liver injury or hepatectomy [7]. Stem cell types that might be used successfully for hepatocyte generation include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), as well as mesenchymal stem cells (MSCs) derived from bone marrow or adipose tissue.

ESCs, which are pluripotent cells derived from human blastocysts, have unlimited proliferation potential and can differentiate into almost any cell or tissue type, including hepatic cells [15]. Cai *et al.* demonstrated efficient hepatic differentiation of human ESCs [16]. These human ESC-derived hepatic cells exhibited characteristics of mature hepatocytes and were successfully engrafted into the liver of mice with CCl_4 injection-induced cirrhosis [16]. Another study performed by Basma *et al.* reported that human ESC-derived hepatocytes expressed hepatocyte-specific markers, secreted human liver-specific proteins, and had cytochrome P450 metabolic activity levels similar to primary human hepatocytes [17]. These human ESC-derived hepatocytes were engrafted into the livers of immune-deficient mice with a 50% partial hepatectomy [17] and significant amounts of human albumin and α1-antitrypsin were detected in the serum isolated from mice receiving the human ESC-derived hepatocytes [17]. Human albumin and cytokeratin 18-expressing cells were also observed in the liver tissue following transplantation.

Human iPSCs were recently highlighted as another potential cell source of hepatocytes for liver tissue engineering. Takahashi *et al*. first derived human iPSCs from dermal fibroblasts using viral transduction of four transcription factors [18]. Since then more cell sources and transcription factor gene delivery strategies have been tested with the goal of improving the efficiency of iPSC induction [19]. Human iPSCs generated from autologous somatic cells by reprogramming could be used as cell therapeutics without the risk of immunogenicity. Additionally, the pluripotency of human iPSCs is comparable to that of human ESCs [19]. Si-Tayeb *et al*. developed a multistage procedure for efficient generation of hepatocyte-like cells from human iPSCs [20]. These cells exhibited liver function and integrated into the hepatic parenchyma *in vivo* [20]. Another study reported that iPSCs generated without viral transduction differentiated into fully mature hepatocytes with a full range of liver functions [21]. The iPSC-derived hepatocytes also showed proliferative capabilities similar to normal hepatocytes and were able to regenerate the liver after a two-thirds partial hepatectomy in mice with a fumarylacetoacetate hydrolase deficiency [21]. More recently, Liu *et al.* evaluated the ability of human iPSCs produced from three different germ layer tissues to undergo hepatic differentiation [22]. These human iPSC lines from different origins differentiated into hepatic lineage cells with an efficiency similar to that of human ESCs, irrespective of their parental epigenetic status [22]. They could also be successfully engrafted into the liver tissue of mice with liver cirrhosis [22] and they secreted human-specific liver proteins into the circulating blood at levels comparable to human primary hepatocytes [22].

Although pluripotent stem cells may be a suitable alternative to hepatocytes for liver regeneration, they have several fundamental limitations due to safety and ethical issues. For example, controlling differentiation is still complicated in these cells and requires multiple steps along with supplementation with various growth factors and

chemical compounds [19]. On the other hand, human ESCs obtained by embryo destruction have ethical issues associated with their use and can produce an immune response if transplanted. These two issues could be avoided by using human iPSCs generated from autologous somatic cells; however, the reprogramming efficiency of iPSC induction is typically low [19]. In addition, the use of viral vectors (retrovirus or lentivirus) may evoke an immune response and mutagenesis [19]. In both pluripotent stem cell types, teratomaformation originating from uncontrolled differentiation or contamination of undifferentiated stem cells is a common concern.

In this regard, adult stem cells such as MSCs may be a more feasible source of hepatocytes for clinical applications. Human MSCs can be easily isolated from autologous sources, such as bone marrow and adipose tissue [6]. *In vitro* expansion of human MSCs is relatively easy compared to pluripotent stem cells [6]. MSCs typically differentiate into mesenchymal lineage cells, including osteoblasts, chondrocytes, and adipocytes [23]. However, they also have the ability to undergo hepatic differentiation with proper media supplementation [24]. Stock *et al.* established a protocol for generating hepatocytes from MSCs isolated from a variety of animal and human tissues [25]. These cells were then engrafted into murine liver tissues [25]. In another study, Li *et al.* developed a novel protocol for generating hepatic lineage cells from MSCs *via* co-culture without growth factor supplementation [26]. Four weeks after transplantation of these co-cultured MSC-derived hepatocyte-like cells into the spleens of rats with liver cirrhosis, fibrosis was remarkably decreased and significant expression of cytokeratin 18 and 19 was observed in the injured livers [26]. Several other researchers using human MSCs derived from bone marrow [27] and adipose tissue [28] also obtained results consistent with the findings of these studies. This demonstrates that hepatocytes can be efficiently generated from MSCs and that functional integration of the transplanted MSC-derived hepatocyte-like cells into the liver tissue in these animal models can be achieved.

2.3. Liver progenitor cells

Liver progenitor cells are another alternative cell source for liver tissue engineering. Liver progenitor cells, generally known as oval cells, are activated for the regeneration of hepatic tissues in response to acute or chronic liver injury [29]. Oval cells express various hepatic lineage markers, such as α-fetoprotein (an early hepatic lineage marker) and albumin (a mature hepatocyte marker) [30,31]. Thus, oval cells may be able to differentiate into mature hepatocytes. In a previous study, transplanted oval cells were shown to have a high proliferative ability and high engraftment capacity in animal models with liver injury, in which 90% of the hepatocytes in the recipient livers were replaced by transplanted oval cells [32].

Hepatic progenitor cells isolated from human fetal livers can differentiate into hepatocytes and repopulate the liver. Human fetal liver serves as a rich source of hepatic progenitor cells [33]. Hepatic progenitor cells contained in human fetal livers are highly proliferative, minimally immunogenic, and resistant to ischemic injury, all of which are considered major advantages to using these cells for liver tissue regeneration [33]. In fact, several groups have already demonstrated the therapeutic potential of human fetal liver-derived progenitor cells in preclinical studies using animals with a hepatectomy [34,35]. More recently, Khan *et al*. demonstrated the efficacy and safety of human fetal liver-derived progenitor cell therapy in patients with end-stage liver cirrhosis [33]. After human fetal liver-derived progenitor cells were infused into 25 patients with liver cirrhosis *via* the hepatic artery, remarkable clinical improvement was observed in all patients who received the progenitor cells 6 months after cell transplantation [33].

3. Biomaterials for Liver Tissue Engineering

The regenerative potential of hepatocytes, hepatic progenitor cells, and stem cells could be improved by using the appropriate biomaterial scaffolds for transplantation into injured regions of the liver. Hepatocytes or hepatic lineage cells easily lose their specific characteristics and exhibit poor viability in traditional 2D culture systems [7]. However, 3D scaffolds fabricated from biocompatible and biodegradable polymers have been shown to enhance cell survival and engraftment by creating a three dimensional extracellular matrix (ECM)-like microenvironment that mimics *in vivo* conditions was shown to promotes cell adherence and growth [7]. Fabrication of biomaterials with the proper mechanical strength and containing various bioactive molecules could allow for the modulation of biochemical and mechanical cues that promote cell proliferation, differentiation, and function of hepatic cells. This could significantly facilitate regeneration of liver tissues. These fabricated scaffolds may also serve to protect the transplanted cells from unfavorable *in vivo* micro environments (*e.g.,* hypoxia or inflammation) in the injured tissues [7]. In this section, the types of biomaterials used in liver tissue engineering will be reviewed, including synthetic/natural polymeric scaffolds, decellularized matrices, and hydrogels (Table 1).

3.1. Synthetic polymer scaffolds

Three-dimensional synthetic polymer scaffolds with porous

Table 1. Biomaterials and cells for liver tissue engineering

Biomaterials		Cells	Models	References
Synthetic polymer scaffold	PLGA disk	Hepatocyte	*In vitro*	[41]
	PLGA disk	Hepatocyte & nonparenchymal liver cell	*In vitro*	[42]
	PLLA scaffold	Hepatocyte	*In vitro*	[43]
	PLLA/PGA scaffold	Embryonic stem cell	*In vitro*	[44]
	Polyamide nanofiber	Bone marrow-derived mesenchymal stem cell	*In vivo* (hepatic fibrosis)	[46]
	Polyamide nanofiber	Embryonic stem cell	*In vitro*	[47]
	PCL nanofiber	Cord blood-derived unrestricted somatic stem cell	*In vitro*	[48]
Natural polymer scaffold	Alginate scaffold	Hepatocyte	*In vitro*	[51]
	Alginate scaffold	Hepatocyte & liver progenitor cell	*In vitro*	[52]
	Alginate scaffold	Bone marrow-derived mesenchymal stem cell	*In vitro*	[53]
	Hyaluronate scaffold	Fetal liver cell	*In vivo* (Wilson's disease)	[56]
	Hyaluronate scaffold	Hepatocyte	*In vivo* (subdermal implantation)	[57]
	Fructose-modified chitosan scaffold	Hepatocyte	*In vitro*	[58]
	Galactosylated chitosan nanofiber	Hepatocyte	*In vitro*	[59]
Decellularized matrix	Decellularized liver matrix	Hepatocyte	*In vivo* (auxiliary liver graft transplantation)	[70]
	Decellularized liver matrix	Hepatocyte	*In vitro*	[72]
	Decellularized liver matrix	Fetal liver cell & endothelial cell	*In vivo* (intra-abdominal ectopic transplantation)	[71]
	Decellularized liver matrix	HepG2 or hepatocyte	*In vitro*	[73]
	Decellularized liver matrix	Hepatic stem cell	*In vitro*	[74]
Hydrogel	PEG hydrogel	Liver progenitor cell	*In vitro*	[75]
	PEG hydrogel	Hepatocyte	*In vitro*	[76]
	PEG hydrogel	Hepatocyte & fibroblast	*In vivo* (intra-peritoneal implantation)	[77]
	Peptide hydrogel	Hepatocyte	*In vitro*	[78,79]

structures could facilitate liver tissue regeneration. Several types of biocompatible and biodegradable polymers, including poly(lactic-co-glycolic acid) (PLGA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), and poly(ε-caprolactone) (PCL) have been used to fabricate 3D scaffolds [36,37]. Importantly, these synthetic polymers have been approved by the Food and Drug Administration for specific applications in the human body [36]. Because these polymers contain ester bonds that are susceptible to hydrolysis, they degrade by non-enzymatic hydrolysis [36]. As a scaffold, they may provide a temporary micro-environment to guide new tissue growth and organization prior to complete degradation. The degradation rate of these polymers can be tailored by altering their molecular weights or the copolymer ratio, and the degradation products of these polymers are eliminated from the body via the respiratory route [36]. The thermoplastic properties of these polymers allow them to be easily processed into desired shapes by various fabrication techniques, such as molding, extrusion, and solvent casting [38-40].

Several studies have found that 3D microporous scaffolds made of PLGA [41,42] or PLLA [43] promote adhesion, proliferation, and maturation of primary hepatocytes. Nonwoven scaffolds composed of PLLA and PGA have also been shown to facilitate hepatic differentiation of mouse ESCs [44]. Using a stepwise addition of growth factors and hormones required for hepatic differentiation, embryoid body-derived cells were able to differentiate into hepatocyte-like cells. The differentiated hepatocyte-like cells on 3D scaffolds expressed liver-specific markers and proteins, and exhibited hepatic functions including albumin secretion and glycogen storage [44].

Recent studies have focused on using nanofibrous scaffolds to enhance hepatocyte proliferation and differentiation through topographical alterations. Electrospinning is a technique that uses an electrical charge to draw fine fibers out of a polymer solution, and it is typically used to fabricate 3D scaffolds with nanofibrous structures. Three-dimensional nanofibrous scaffolds can facilitate hepatic differentiation of a variety of stem cells [45-48]. For instance, bone marrow-derived MSCs cultured on nanofibrous scaffolds composed of PCL, collagen, and polyethersulfone

that were fabricated by electrospinning expressed hepatocyte specific markers, including albumin, α-fetoprotein, cytokeratin-18, cytokeratin-19, and cytochrome P450 [45]. The density of albumin-positive cells was much higher on the nanofibrous scaffolds when compared with cells in 2D culture [45]. Piryaei *et al.* also used electrospun nanofibrous scaffolds made of polyamide for the hepatic differentiation of bone marrow-derived MSCs [46]. Urea production, albumin secretion, and metabolic activity of the cytochrome P450 enzymes were significantly increased in differentiated hepatic-like cells cultured on electrospun nanofibrous scaffolds during long-term culture (up to 36 days after seeding) [46]. Upon intravenous transplantation in mice with liver fibrosis, MSC-derived hepatic-like cells obtained from culture with nanofibrous scaffolds were able to differentiate into functional hepatocytes and reduce liver fibrosis [46]. Other stem cell types including human ESCs [47] and cord blood-derived stem cells [48] demonstrated enhanced hepatic differentiation and function when cultured on 3D nanofibrous polymer scaffolds. These results demonstrate that the nano-scale porous, aligned structures of nanofibrous scaffolds mimic *in vivo* topographical microenvironments and may be appropriate scaffolding systems for hepatic lineage cells.

Synthetic polymer scaffolds manufactured and reproducibly fabricated as mentioned earlier would lack endogenous factors that modulate cellular behavior [49]. Thus, these materials may not provide binding sites that activate integrins and other cell surface receptors [49]. Modification with ECM proteins or peptides (*e.g.*, Arg-Gly-Asp; RGD) may improve cell function on the synthetic polymer scaffolds. Additionally, synthetic polymers have some disadvantages, including potential toxicity from acidic degradation products and a lack of functional groups available for covalent modification, which may limit their usefulness in biomedical applications [36].

3.2. Natural polymer scaffolds

Three-dimensional scaffolds fabricated with naturally derived polymers could enhance hepatic lineage cellular activity and function. Because natural polymers contain numerous integrin-binding sites and growth factors, scaffolds composed of natural polymers may stimulate signaling cascades involved in cell proliferation and/or differentiation [49]. Polymers prepared from natural sources and used for liver tissue engineering include alginate, hyaluronate, and chitosan.

Alginate, which is an anionic polysaccharide extracted from brown algae, is one of the most widely used natural biomaterials for tissue engineering and drug delivery. Alginate has several advantages as a biomaterial for tissue engineering applications, including biocompatibility, low toxicity, low cost, and simple gelation with divalent cations ($Ca^{2+}$and Mg^{2+}) [50]. Dvir-Ginzberg *et al.* examined the hepatocyte seeding efficiency and proliferation within porous alginate scaffolds [51]. This work revealed that hepatocyte functions, such as albumin and urea secretion, and detoxification by cytochrome P450 and phase II conjugating enzyme activities, were maintained during a 1-week culture period [51]. They also used alginate scaffolds to culture cells isolated from newborn rat liver, a heterogeneous population of hepatocytes and progenitors [52]. Within 3 days, the cells seeded onto the alginate scaffolds formed compact spheroids. These spheroids developed into functional hepatic tissues with mature hepatocytes after 6 weeks in culture [52]. Alginate scaffolds were also tested for their ability to induce hepatic differentiation of bone marrow stem cells. Lin *et al.* cultured bone marrow stem cells in alginate scaffolds in the presence of specific growth factors, including hepatocyte growth factor (HGF), epidermal growth factor, and fibroblast growth factor-4 [53]. Bone marrow stem cells in alginate scaffolds differentiated into hepatocyte-like cells, which was assessed based on the expression of liver-specific markers (α-fetoprotein, albumin, and cytokeratin 18) and newly acquired hepatic functions (albumin/urea production and glycogen storage) [53].

Hyaluronate is a glycosaminoglycan component of the ECM that is distributed widely throughout connective, epithelial, and neural tissues [50]. Hyaluronate contributes significantly to cell proliferation and migration, and plays a significant role in wound healing [50,54]. Hyaluronate is degraded by hyaluronidase, which is an enzyme found in cells and serum. Given that hepatocytes contain intracellular-specific binding sites for hyaluronate [55], this natural polymer may promote the hepatocellular function of liver cells *via* signaling pathways initiated by cell binding to surface receptors. In fact, several studies have demonstrated the benefits of using hyaluronate scaffolds in liver tissue engineering [56,57]. Katsuda *et al*. transplanted fetal liver-derived cells in hyaluronate sponge scaffolds into a rat model of Wilson's disease, a genetic disorder in which copper accumulates in tissues resulting in liver disease [56]. This approach prevented jaundice and significantly reduced blood copper concentrations. Accordingly, a decrease in total serum bilirubin and an increase in albumin production were observed in animals that recieved the liver cell-seeded constructs [56]. Another study demonstrated the usefulness of hyaluronate scaffolds for long-term culture and *in vivo* transplantation of hepatocytes [57]. In this study, hyaluronate scaffolds enriched with ECM components allowed for long-term maintenance of hepatocyte function (albumin secretion) *in vitro*, and efficient generation/organization of hepatic aggregates *in vivo* [57].

Chitosan is a linear polysaccharide composed of randomly

distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine residues [50]. Chitosan is usually produced by N-deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans and in the cell walls of fungi [50]. Due to its biocompatibility, low toxicity, and structural similarity to natural glycosaminoglycans, chitosan has been used in a number of biomedical applications, including tissue engineering [50]. The enzymes chitosanase and lysozyme degrade chitosan. The modification of chitosan with polysaccharide residues (fructose and galactose) that bind to receptors on liver cells may enhance cell behavior on chitosan scaffolds, which would be important to liver tissue engineering applications. In fact, Li *et al.* showed that fructose-modified chitosan scaffolds enhanced the metabolic activity of rat hepatocytes (albumin secretion and urea synthesis) when compared with unmodified chitosan scaffolds [58]. Similarly, primary rat hepatocytes cultured on electrospun nanofibrous scaffolds fabricated from galactosylated chitosan exhibited superior liver-specific function in terms of albumin secretion, urea synthesis, and cytochrome P450 enzyme activity [59].

Scaffolds made of naturally derived polymers were able to enhance the cellular activity required for hepatic tissue regeneration. However, controlling the rate and mode of degradation is difficult in natural polymeric scaffolds when compared with synthetic polymer scaffolds [50]. Additionally, natural polymeric scaffolds do not have the desired mechanical properties of hepatic tissues and the mechanical properties of these scaffolds need to be improved in order to make naturally derived polymers a viable option for liver tissue engineering applications.

3.3. Decellularized matrices

Hepatocytes and hepatic linage cells require 3D scaffolds with perfect ECM structures in order to maintain their hepatic stability and liver-specific functions. These scaffolds also require specific mechanical properties. Therefore, mimicking the architecture and mechanical properties of the native liver is critical in the fabrication of liver tissue engineered scaffolds. Despite the many advantages of synthetic and natural polymer-based scaffolds, the production of transplantable and functional liver tissue using these materials still remains a challenge.

Decellularization is an attractive technique for preparing scaffolds in tissue engineering applications. Decellularized materials are biological matrices prepared from native organs or tissues through a process that removes cellular components. Decellularized matrices can be prepared by using ionic or nonionic detergents, such as Triton X-100 and sodium dodecyl sulfate, or by enzymatic treatment with DNase, RNase, trypsin, and/or collagenase [60]. The cells are eliminated from the organ by the decellularization process; however, the ECMs and other structural molecules remain. Thus, decellularized matrices potentially retain the architecture of the original tissue, including the native microvasculature [60]. Decellularized matrices facilitate cell attachment, migration, and differentiation, which ultimately leads to functional regeneration of the organs. The mechanical properties of decellularized matrices are similar to those of native tissues [60,61]. The immunogenicity of decellularized tissue matrices is low because cellular antigens that evoke an immune response are removed from the tissues [60,62]. Decellularized matrices show potential in many areas of tissue engineering and have been used for regeneration of many tissues, including the heart [63], bladder [64], arteries [65-67], cartilage [68], and trachea [69].

The 3D ECM architecture of the liver along with a functional microvascular networks can be preserved in these decellularized matrices. Preservation of these structures may provide crucial cues for the engraftment, survival, and long-term function of hepatocytes. Several recent studies that created decellularized liver matrices have reported a subsequent recellularization strategy for liver tissue engineering. Uygun *et al*. developed a whole-liver decellularization technique by portal perfusion with sodium dodecyl sulfate to remove cellular components followed by perfusion-seeding and culture techniques for the preparation of recellularized liver matrix for transplantation [70]. The decellularization process employed in this study preserved major liver-specific ECM components in the matrices, including collagen I and IV, fibronectin, and laminin [70]. Most importantly, the decellularization procedure preserved the structural and functional characteristics of the original microvascular network, which allowed for efficient recellularization of the liver matrix with adult hepatocytes *via* perfusion for *in vitro* culture [70].

Considering that the construction of a functional vascular network has been a key issue in the development of functional tissue-engineered livers, decellularized liver matrices with intact vascular networks could be used as scaffolding platforms for efficiently delivering a supply of nutrients and oxygen to the seeded hepatocytes [70]. This may result in enhanced engraftment, long-term survival, and hepatic function of hepatocytes. In fact, recellularized liver matrices have been shown to support hepatic function, including albumin secretion, urea synthesis, and cytochrome P450 expression at levels comparable to a normal liver *in vitro* [70]. Retention of vascular structures allowed for efficient transplantation of recellularized liver grafts in rats, and post-transplantation analysis demonstrated hepatocyte survival and function with minimal ischemic damage [70].

Other studies have also demonstrated the advantages of using decellularized whole liver matrices in liver tissue engineering. Recently, Baptista *et al.* developed a similar

decellularization procedure using detergent perfusion to selectively remove the cellular components of the tissue while preserving the ECM components and the intact vascular network [71]. Human fetal liver cells and endothelial cells were repopulated within the decellularized liver matrices and expressed typical endothelial, hepatic, and biliary epithelial markers in the matrices, indicating that liver-like tissue was generated by the constructs *in vitro* [71]. Soto-Gutierrez *et al*. used a combination of enzymatic, detergent, and mechanical treatment to remove all cellular components from rat livers [72]. They evaluated the effects of the three different methods on hepatocyte seeding in the decellularized liver matrices to maximize cell survival and function (direct parenchymal injection, multistep infusion, and continuous perfusion).The multistep infusion method generated ~90% hepatocyte engraftment and supported liver-specific functions of the engrafted hepatocytes, including albumin production, urea metabolism, and cytochrome P450 induction [72].

Decellularized liver matrices induce rapid and efficient hepatic lineage differentiation of stem cells. A previous study demonstrated that liver tissue-derived ECMs may provide the optimal substrate for hepatocyte culture, indicating that tissue-specific ECM compositions can modulate cellular interactions in a lineage-specific manner [73]. Thus, lineage-specific differentiation of stem cells might be directed by tissue-specific ECM components. In fact, Wang *et al.* applied this strategy in order to efficiently differentiate human hepatic stem cells [74]. They prepared decellularized liver matrices using a novel perfusion protocol with phospholipase A2, nucleases (DNase and RNase), and a mild detergent (sodium deoxycholate) [74]. The resulting scaffolds retained the native vasculature, liver-specific architecture, many matrix components (collagen, laminin, fibronectin, elastin, proteoglycans, and nidogen/entactin), as well as physiological levels of matrix-bound growth factors and cytokines [74]. These liver-specific matrix components and matrix bound factors facilitated human hepatic stem cell differentiation into mature, functional parenchymal cells, and maintained a stable phenotype for more than 8 weeks [74].

3.4. Hydrogels

Injectable hydrogels may be useful for efficient and minimally invasive cell transplantation, especially for defects that are irregular in shape because of their *in situ* polymerization. Various types of hydrogel systems have been prepared from synthetic or natural polymers and applied as scaffold platforms for hepatocyte culture and transplantation.

The poly(ethylene glycol) (PEG) hydrogel is a widely used synthetic hydrogel for tissue engineering applications because of its tunable mechanical properties and efficient modification of bioactive molecules, such as peptides [75]. PEG-based hydrogels can be cross-linked via a photo-polymerization process that will efficiently encapsulate the cells for transplantation [75]. Several studies have demonstrated the utility of PEG hydrogels as 3D scaffolds for liver tissue engineering. Underhill *et al*. used photo-polymerizable PEG hydrogels for 3D encapsulation of mouse embryonic liver cells to maintain the survival and hepatocellular function of liver cells [75]. Changing the hydrogel characteristics, including PEG molecular weight and PEG chain length, could control the phenotype and function of the encapsulated liver cells [75]. Interestingly, conjugating adhesive peptides (RGD) to the hydrogel network further improved the hepatic function of liver cells within PEG hydrogels [75]. RGD-conjugated PEG hydrogels maintained long-term enhancement of functional characteristics, including albumin secretion and urea synthesis, in liver cells, highlighting the importance of adhesive peptide-mediated interactions in hepatocyte stability within a 3D construct [75]. Itle *et al.* also examined the effects of PEG hydrogel composition on albumin and fibronectin production by hepatocytes [76]. They revealed that the addition of RGD to PEG hydrogels significantly enhanced the production of these proteins [76]. In a more recent study, *in vivo* tissue-engineered bioartificial livers were developed by encapsulating hepatocytes and fibroblasts (supportive stromal cells) using PEG hydrogels with RGD peptides [77].

Peptide-derived hydrogels are promising materials for hepatocyte encapsulation. Many groups have tested self-assembling peptide hydrogels, which provide stable, highly organized nanofibrous scaffolds, as synthetic ECMs for tissue engineering scaffolds. Self-assembling peptides typically consist of ionic, self-complementary sequences with alternating hydrophobic and hydrophilic domains [78]. The geometry of the resulting peptide scaffolds can be manipulated by modulating the spacing of charged and hydrophobic residues in the amino acid sequence [78]. Cell adhesive sequences, such as RGD, can also be introduced into the peptide hydrogels to enhance cell attachment and migration. Wang *et al*. demonstrated the utility of a self-assembling peptide hydrogel, named "PuraMatrix", for culturing primary hepatocytes [78]. The peptide sequence in PuraMatrix was shown to facilitate cell attachment and migration, which enabled the rapid formation of cell-cell contacts [78]. This hydrogel quickly generated primary hepatocyte spheroids within 3 days of seeding [78]. Albumin and urea secretion, and cytochrome P450 activity of the hepatocytes cultured with PuraMatrix were maintained at high levels throughout the culture period [78]. Another study demonstrated that self-assembling peptide hydrogels functionalized with laminin receptor ligands enhanced the

gene expression of albumin, hepatocyte nuclear factor 4α, and tyrosine aminotransferase [79].

4. Growth Factor Delivery for Liver Tissue Engineering

Despite advances in liver tissue engineering by using diverse types of cells and scaffolds, engineering approaches are often limited by poor engraftment of the engineered hepatic tissues due to insufficient vascularization. Delivery of growth factors that promote angiogenesis and hepatic cell proliferation could provide a strategy to overcome this limitation. Conjugation or encapsulation of growth factors into 3D scaffolds should mediate sustained and controlled delivery of growth factors. Delivery systems for growth factors to improve liver tissue engineering will be discussed in this section (Table 2).

4.1. Delivery of angiogenic growth factors

The delivery of angiogenic growth factors, such as vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF), using biodegradable polymer scaffolds has been shown to improve angiogenesis and hepatocyte engraftment. Several studies demonstrated controlled and sustained delivery of VEGF, one of the most potent growth factors in stimulating angiogenic pathways, during hepatocyte transplantation. Kedem *et al*. proposed a strategy of pre-vascularization using VEGF delivery prior to hepatocyte transplantation [80]. Biodegradable PLGA microspheres loaded with human VEGF were incorporated in porous alginate scaffolds and then implanted into the liver lobes of rats. Hepatocytes were transplanted into the regions containing the implanted scaffolds 1 week following pre-vascularization. Pre-implantation of VEGF-releasing scaffolds greatly increased the blood vessel density at the implantation sites and consequently improved hepatocyte engraftment when compared with control scaffolds lacking VEGF [80].

Heparin-conjugated hydrogels or nanoparticles provide efficient platforms for sustained delivery of growth factors that have heparin-binding affinity (*e.g.*, VEGF, bFGF, and HGF). Jeon *et al.* reported that the addition of heparin to a fibrin gel could retard the release of bFGF from the fibrin gel through specific interactions between heparin, bFGF, and fibrin [81]. Almost all of the bFGF was released from the fibrin gels lacking heparin within the first 4 days; however, the release of bFGF from the fibrin gels containing heparin was sustained for up to 7 days *in vitro* [81]. The authors of this work also demonstrated that heparin-conjugated PLGA nanoparticles could be used to control bFGF delivery [82]. The release of bFGF from heparin-conjugated PLGA nanoparticles was sustained for 3 weeks without an initial burst release. The bFGF release was further extended to more than 4 weeks by suspending the heparin-conjugated PLGA nanoparticles in a fibrin gel [82]. When these bFGF delivery systems were applied along with stem cell therapy (cord blood stem cells and adipose-derived stem cells) for ischemic disease treatment, they significantly improved angiogenesis and the *in vivo* survival of transplanted stem cells, thereby improving the therapeutic efficacy of treating the hindlimb [83,84] or myocardial ischemia [85]. In a liver tissue engineering application, Hou *et al.* utilized a heparin-immobilized collagen gel system as a pre-vascularization strategy with VEGF delivery [86]. VEGF with a heparin-binding domain was efficiently entrapped in collagen gels by binding to heparin molecules, and was released in a controlled and sustained manner from the gels [86]. The *in vivo* viability of hepatocytes was significantly improved by pre-vascularization using this heparin-gel system loaded with VEGF [86].

Biodegradable polymer scaffolds with incorporated bFGF have also been used for hepatocyte transplantation. Lee *et al.* examined the effect of local bFGF delivery on angiogenesis and hepatocyte engraftment within tissue-engineered liver constructs [87]. Porous PLLA sponges were fabricated and coated with bFGF, sucralfate, and Hydron [87]. In this system, sucralfate binds to bFGF and protects it from degradation. The Hydron can be used as a delivery vehicle for the mixture of sucralfate-bFGF. The bFGF-coated PLLA scaffolds seeded with primary rat hepatocytes were implanted into the small bowel mesentery of rats. Two weeks after implantation, microvessel density and hepatocyte engraftment were substantially increased in the bFGF-coated scaffold group when compared with the control scaffold group without the bFGF coating [87].

4.2. Delivery of hepatocyte growth factor (HGF)

HGF is involved in a wide range of signal transduction

Table 2. Growth factor delivery systems for liver tissue engineering

Delivery systems	Growth factors	Models	References
PLGA microsphere & alginate scaffold	VEGF	*In vivo* (implantation into liver lobes)	[80]
Heparin-immobilized collagen gel	VEGF	*In vivo* (subcutaneous implantation)	[86]
PLLA sponge	bFGF	*In vivo* (implantation into small bowel mesentery)	[87]
Heparin-immobilized collagen film or gel	HGF	*In vitro* (hepatocyte culture)	[88]
Heparin-conjugated PEG hydrogel	HGF	*In vitro* (hepatocyte culture)	[89]

events including mitogenesis and morphogenesis [88]. In particular, HGF is a potent mitogen for hepatocytes, and is a hepatotropic factor for liver regeneration [88]. Recently, Hou *et al*. developed a sustained HGF delivery system for liver tissue engineering applications [88]. They developed a HGF/heparin-immobilized collagen film or gel system for hepatocyte culture *in vitro*. HGF was immobilized to collagen *via* its high binding affinity for heparin. Hepatocytes in the HGF/heparin-immobilized collagen systems exhibited high levels of albumin synthesis in both 2D film culture and in 3D gel culture and these levels were sustained for more than 2 weeks [88].

In another study, Kim *et al.* described using a heparin-conjugated PEG hydrogel for primary hepatocyte culture [89]. The heparin-conjugated PEG hydrogel resulted in high levels of albumin and urea synthesis by primary hepatocytes for up to 3 weeks in culture [89]. The authors introduced HGF into the heparin-PEG hydrogel system to further improve hepatocellular functions. This system allowed for the controlled release of 40% of the loaded HGF for up to 30 days in culture [89]. Importantly, hepatocytes cultured in HGF-loaded PEG hydrogels maintained significantly higher levels of albumin and urea synthesis than hepatocytes in the PEG hydrogels without HGF [89].

5. Conclusion

In summary, the regenerative capacity of tissue-engineered livers can be improved by the proper combination of cells, biomaterials, and growth factor delivery systems. Functional tissue-engineered livers may be used as transplantable bioartificial livers, which could solve the donor shortage problem. Engineered liver tissues with hepatic functionality may also provide useful surrogates to native livers or hepatocytes in drug screening, drug metabolism, and toxicology studies.

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References

1. Lundquist, F., N. Tygstrup, K. Winkler, K. Mellemgaard, and S. Munck-Petersen (1962) Ethanol metabolism and production of free acetate in the human liver. *J. Clin. Invest.* 41: 955-961.
2. Lobley, G. E., A. Connell, M. A. Lomax, D. S. Brown, E. Milne, A. G. Calder, and D. A. Farningham (1995) Hepatic detoxification of ammonia in the ovine liver: Possible consequences for amino acid catabolism. *Br. J. Nutr.* 73: 667-685.
3. Moshage, H. J., J. A. Janssen, J. H. Franssen, J. C. Hafkenscheid, and S. H. Yap (1987) Study of the molecular mechanism of decreased liver synthesis of albumin in inflammation. *J. Clin. Invest.* 79: 1635-1641.
4. Schwander, J. C., C. Hauri, J. Zapf, and E. R. Froesch (1983) Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused rat liver: Dependence on growth hormone status. *Endocrinol.* 113: 297-305.
5. Michalopoulos, G. K. (2010) Liver regeneration after partial hepatectomy: Critical analysis of mechanistic dilemmas. *Am. J. Pathol.* 176: 2-13.
6. Kisseleva, T., E. Gigante, and D. A. Brenner (2010) Recent advances in liver stem cell therapy. *Curr. Opin. Gastroenterol.* 26: 395-402.
7. Zhang, W., L. Tucker-Kellogg, B. C. Narmada, L. Venkatraman, S. Chang, Y. Lu, N. Tan, J. K. White, R. Jia, S. S. Bhowmick, S. Shen, C. F. Dewey. Jr., and H. Yu (2010) Cell-delivery therapeutics for liver regeneration. *Adv. Drug Deliv. Rev.* 62: 814-826.
8. Mito, M., M. Kusano, and Y. Kawaura (1992) Hepatocyte transplantation in man. *Transplant. Proc.* 24: 3052-3053.
9. Habibullah, C. M., I. H. Syed, A. Qamar, and Z. Taher-Uz (1994) Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. *Transplantation* 58: 951-952.
10. Strom, S. C., R. A. Fisher, M. T. Thompson, A. J. Sanyal, P. E. Cole, J. M. Ham, and M. P. Posner (1997) Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. *Transplantation* 63: 559-569.
11. Kobayashi, N., H. Noguchi, T. Watanabe, T. Matsumura, T. Totsugawa, T. Fujiwara, K. Westerman, P. Leboulch, I. J. Fox, and N. Tanaka (2000) Establishment of a tightly regulated human cell line for the development of hepatocyte transplantation. *Hum. Cell* 13: 7-13.
12. Terry, C. and R. D. Hughes (2009) An optimised method for cryopreservation of human hepatocytes. *Methods Mol. Biol.* 481: 25-34.
13. Terry, C., R. R. Mitry, S. C. Lehec, P. Muiesan, M. Rela, N. D. Heaton, R. D. Hughes, and A. Dhawan (2005) The effects of cryopreservation on human hepatocytes obtained from different sources of liver tissue. *Cell Transplant.* 14: 585-594.
14. Sgroi, A., V. Serre-Beinier, P. Morel, and L. Bühler (2009) What clinical alternatives to whole liver transplantation? Current status of artificial devices and hepatocyte transplantation.*Transplantation* 87: 457-466.
15. Thomson, J. A., J. Itskovitz-Eldor, S. S. Shapiro, M. A. Waknitz, J. J. Swiergiel, V. S. Marshall, and J. M. Jones (1998) Embryonic stem cell lines derived from human blastocysts. *Science* 282: 1145-1147.
16. Cai, J., Y. Zhao, Y. Liu, F. Ye, Z. Song, H. Qin, S. Meng, Y. Chen, R. Zhou, X. Song, Y. Guo, M. Ding, and H. Deng (2007) Directed differentiation of human embryonic stem cells into functional hepatic cells. *Hepatol.* 45: 1229-1239.
17. Basma, H., A. Soto-Gutiérrez, G. R. Yannam, L. Liu, R. Ito, T. Yamamoto, E. Ellis, S. D. Carson, S. Sato, Y. Chen, D Muirhead, N. Navarro-Álvarez, R. J. Wong, J. Roy-Chowdhury, J. L. Platt, D. F. Mercer, J. D. Miller, S. C. Storm, N. Kobayashi, and I. J. Fox (2009) Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. *Gastroenterol.* 136: 990-999.
18. Takahashi, K., K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, and S. Yamanaka (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131: 861-872.
19. Hanna, J. H., K. Saha, and R. Jaenisch (2010) Pluripotency and cellular reprogramming: Facts, hypotheses, unresolved issues. *Cell* 143: 508-525.

20. Si-Tayeb, K., F. K. Noto, M. Nagaoka, J. Li, M. A. Battle, C. Duris, P. E. North, S. Dalton, and S. A. Duncan (2010) Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. *Hepatol.* 51: 297-305.
21. Espejel, S., G. R. Roll, K. J. McLaughlin, A. Y. Lee, J. Y. Zhang, D. J. Laird, K. Okita, S. Yamanaka, and H. Willenbring (2010) Induced pluripotent stem cell-derived hepatocytes have the functional and proliferative capabilities needed for liver regeneration in mice. *J. Clin. Invest.* 120: 3120-3126.
22. Liu, H., Y. Kim, S. Sharkis, L. Marchionni, and Y. Y. Jang (2011) *In vivo* liver regeneration potential of human induced pluripotent stem cells from diverse origins. *Sci. Transl. Med.* 3: 82ra39.
23. Pittenger, M. F., A. M. Mackay, S. C. Beck, R. K. Jaiswal, R. Douglas, J. D. Mosca, M. A. Moorman, D. W. Simonetti, S. Craig, and D. R. Marshak (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143-147.
24. Li, T. Z., S. H. Shin, H. H. Cho, J. H. Kim, and H. Suh (2008) Growth factor-free cultured rat bone marrow derived mesenchymal stem cells towards hepatic progenitor cell differentiation. *Biotechnol. Bioproc. Eng.* 13: 659-665.
25. Stock, P., S. Brückner, S. Ebensing, M. Hempel, M. M. Dollinger, and B. Christ (2010) The generation of hepatocytes from mesenchymal stem cells and engraftment into murine liver. *Nat. Protoc.* 5: 617-627.
26. Li, T. Z., J. H. Kim, H. H. Cho, H. S. Lee, K. S. Kim, S. W. Lee, and H. Suh (2010) Therapeutic potential of bone-marrow-derived mesenchymal stem cells differentiated with growth-factor-free coculture method in liver-injured rats. *Tissue Eng. Part A* 16: 2649-2659.
27. Aurich, I., L. P. Mueller, H. Aurich, J. Luetzkendorf, K. Tisljar, M. M. Dollinger, W. Schormann, J. Walldorf, J. G. Hengstler, W. E. Fleig, and B. Christ (2007) Functional integration of hepatocytes derived from human mesenchymal stem cells into mouse livers. *Gut.* 56: 405-415.
28. Aurich, H., M. Sgodda, P. Kaltwasser, M. Vetter, A. Weise, T. Liehr, M. Brulport, J. G. Hengstler, M. M. Dollinger, W. E. Fleig, and B. Christ (2009) Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue *in vitro* promotes hepatic integration *in vivo*. *Gut.* 58: 570-581.
29. Pi, L., S. H. Oh, T. Shupe, and B. E. Petersen (2005) Role of connective tissue growth factor in oval cell response during liver regeneration after 2-AAF/PHx in rats. *Gastroenterol.* 128: 2077-2088.
30. Lemire, J. M., N. Shiojiri, and N. Fausto (1991) Oval cell proliferation and the origin of small hepatocytes in liver injury induced by D-galactosamine. *Am. J. Pathol.* 139: 535-552.
31. Wang, X., M. Foster, M. Al-Dhalimy, E. Lagasse, M. Finegold, and M. Grompe (2003) The origin and liver repopulating capacity of murine oval cells. *Proc. Natl. Acad. Sci. USA* 100 Suppl 1: 11881-11888.
32. Yovchev, M. I., P. N. Grozdanov, H. Zhou, H. Racheria, C. Guha, and M. D. Dabeva (2008) Identification of adult hepatic progenitor cells capable of repopulating injured rat liver. *Hepatol.* 47: 636-647.
33. Khan, A. A., M. V. Shaik, N. Parveen, A. Rajendraprasad, M. A. Aleem, M. A Habeeb, G. Srinivas, T. A. Raj, S. K. Tiwari, K. Kumaresan, J. Venkateswarlu, G. Pande, and C. M. Habibullah (2010) Human fetal liver-derived stem cell transplantation as supportive modality in the management of end-stage decompensated liver cirrhosis. *Cell Transplant.* 19: 409-418.
34. Oertel, M., A. Menthena, Y. Q. Chen, B. Teisner, C. H. Jensen, and D. A. Shafritz (2008) Purification of fetal liver stem/progenitor cells containing all the repopulation potential for normal adult rat liver. *Gastroenterol.* 134: 823-832.
35. Sandhu, J. S., P. M. Petkov, M. D. Dabeva, and D. A. Shafritz (2001) Stem cell properties and repopulation of the rat liver by fetal liver epithelial progenitor cells. *Am. J. Pathol.* 159: 1323-1334.
36. Kim, B. S. and D. J. Mooney (1998) Development of biocompatible synthetic extracellular matrices for tissue engineering. *Trends Biotechnol.* 16: 224-230.
37. Lee, N. K., H. J. Oh, C. M. Hong, H. Suh, and S. H. Hong (2009) Comparison of the synthetic biodegradable polymers, polylactide (PLA), and polylactic-co-glycolic acid (PLGA) as scaffolds for artificial cartilage. *Biotechnol. Bioproc. Eng.* 14: 180-186.
38. Cho, S. W., S. S. Kim, J. W. Rhie, H. M. Cho, C. Y. Choi, and B. S. Kim (2005) Engineering of volume-stable adipose tissues. *Biomat.* 26: 3577-3585.
39. Cho, S. W., O. Jeon, J. E. Lim, S. J. Gwak, S. S. Kim, C. Y. Choi, D. I. Kim, and B. S. Kim (2006) Preliminary experience with tissue engineering of a venous vascular patch by using bone marrow-derived cells and a hybrid biodegradable polymer scaffold. *J. Vasc. Surg.* 44: 1329-1340.
40. Cho, S. W., K. W. Song, J. W. Rhie, M. H. Park, C. Y. Choi, and B. S. Kim (2007) Engineered adipose tissue formation enhanced by basic fibroblast growth factor and a mechanically stable environment. *Cell Transplant.* 16: 421-434.
41. Hasirci, V., F. Berthiaume, S. P. Bondre, J. D. Gresser, D. J. Trantolo, M. Toner, and D. L. Wise (2001) Expression of liver-specific functions by rat hepatocytes seeded in treated poly(lactic-co-glycolic) acid biodegradable foams. *Tissue Eng.* 7: 385-394.
42. Kim, S. S., H. Utsunomiya, J. A. Koski, B. M. Wu, M. J. Cima, J. Sohn, K. Mukai, L. G. Griffith, and J. P. Vacanti (1998) Survival and function of hepatocytes on a novel three-dimensional synthetic biodegradable polymer scaffold with an intrinsic network of channels. *Ann. Surg.* 228: 8-13.
43. Huang, H., S. Hanada, N. Kojima, and Y. Sakai (2006) Enhanced functional maturation of fetal porcine hepatocytes in three-dimensional poly-L-lactic acid scaffolds: A culture condition suitable for engineered liver tissues in large-scale animal studies. *Cell Transplant.* 15: 799-809.
44. Liu, T., S. Zhang, X. Chen, G. Li, and Y. Wang (2010) Hepatic differentiation of mouse embryonic stem cells in three-dimensional polymer scaffolds. *Tissue Eng. Part A* 16: 1115-1122.
45. Kazemnejad, S., A. Allameh, M. Soleimani, A. Gharehbaghian, Y. Mohammadi, N. Amirizadeh, and M. Jazayery (2009) Biochemical and molecular characterization of hepatocyte-like cells derived from human bone marrow mesenchymal stem cells on a novel three-dimensional biocompatible nanofibrous scaffold. *J. Gastroenterol. Hepatol.* 24: 278-287.
46. Piryaei, A., M. R. Valojerdi, M. Shahsavani, and H. Baharvand (2011) Differentiation of bone marrow-derived mesenchymal stem cells into hepatocyte-like cells on nanofibers and their transplantation into a carbon tetrachloride-induced liver fibrosis model. *Stem Cell Rev.* 7: 103-118.
47. Farzaneh, Z., B. Pournasr, M. Ebrahimi, N. Aghdami, and H. Baharvand (2010) Enhanced functions of human embryonic stem cell-derived hepatocyte-like cells on three-dimensional nanofibrillar surfaces. *Stem Cell Rev.* 6: 601-610.
48. Hashemi, S. M., M. Soleimani, S. S. Zargarian, V. Haddadi-Asi, N. Ahmadbeigi, S. Soudi, Y. Gheisari, A. Hajarizadeh, and Y. Mohammadi (2009) *In vitro* differentiation of human cord blood-derived unrestricted somatic stem cells into hepatocyte-like cells on poly(epsilon-caprolactone) nanofiber scaffolds. *Cells Tissues Organs* 190: 135-149.
49. Tibbitt, M. W. and K. S. Anseth (2009) Hydrogels as extracellular matrix mimics for 3D cell culture. *Biotechnol. Bioeng.* 103: 655-663.
50. Lee, K. Y. and D. J. Mooney (2001) Hydrogels for tissue engineering. *Chem. Rev.* 101: 1869-1879.
51. Dvir-Ginzberg, M., I. Gamlieli-Bonshtein, R. Agbaria, and S. Cohen (2003) Liver tissue engineering within alginate scaffolds:

Effects of cell-seeding density on hepatocyte viability, morphology, and function. *Tissue Eng.* 9: 757-766.
52. Dvir-Ginzberg, M., T. Elkayam, and S. Cohen (2008) Induced differentiation and maturation of newborn liver cells into functional hepatic tissue in macroporous alginate scaffolds. *FASEB J.* 22: 1440-1449.
53. Lin, N., J. Lin, L. Bo, P. Weidong, S. Chen, and R. Xu (2010) Differentiation of bone marrow-derived mesenchymal stem cells into hepatocyte-like cells in an alginate scaffold. *Cell Prolif.* 43: 427-434.
54. Seo, Y. K., J. K. Park, K. Y. Song, S. Y. Kwon, and H. S. Lee (2010) Wound healing effect of collagen-hyaluronic acid implanted in partially injured anterior cruciate ligament of dog. *Biotechnol. Bioproc. Eng.*15: 552-558.
55. Frost, S. J., R. H. Raja, and P. H. Weigel (1990) Characterization of an intracellular hyaluronic acid binding site in isolated rat hepatocytes. *Biochem.* 29: 10425-10432.
56. Katsuda, T., T. Teratani, T. Ochiya, and Y. Sakai (2010) Transplantation of a fetal liver cell-loaded hyaluronic acid sponge onto the mesentery recovers a Wilson's disease model rat. *J. Biochem.* 148: 281-288.
57. Zavan, B., P. Brun, V. Vindigni, A. Amadori, W. Habeler, P. Pontisso, D. Montemurro, G. Abatangelo, and R. Cortivo (2005) Extracellular matrix-enriched polymeric scaffolds as a substrate for hepatocyte cultures: *In vitro* and *in vivo* studies. *Biomat.* 26: 7038-7045.
58. Li, J., J. Pan, L. Zhang, and Y. Yu (2003) Culture of hepatocytes on fructose-modified chitosan scaffolds. *Biomat.* 24: 2317-2322.
59. Feng, Z. Q., X. Chu, N. P. Huang, T. Wang, Y. Wang, X. Shi, Y. Ding, and Z. Z. Gu (2009) The effect of nanofibrous galactosylated chitosan scaffolds on the formation of rat primary hepatocyte aggregates and the maintenance of liver function. *Biomat.* 30: 2753-2763.
60. Schmidt, C. E. and J. M. Baier (2000) Acellular vascular tissues: Natural biomaterials for tissue repair and tissue engineering. *Biomat.* 21: 2215-2231.
61. Tamura, N., T. Nakamura, H. Terai, A. Iwakura, S. Nomura, Y. Shimizu, and M. Komeda (2003) A new acellular vascular prosthesis as a scaffold for host tissue regeneration. *Int. J. Artif. Organs* 26: 783-792.
62. Hawkins, J. A., N. D. Hillman, L. M. Lambert, J. Jones, G. B. Di Russo, T. Profaizer, T. C. Fuller, L. L. Minich, R. V. Williams, and R. E. Shaddy (2003) Immunogenicity of decellularized cryopreserved allografts in pediatric cardiac surgery: Comparison with standard cryopreserved allografts. *J. Thorac. Cardiovasc. Surg.* 126: 247-252.
63. Ott, H. C., T. S. Matthiesen, S. K. Goh, L. D. Black, S. M. Kren, T. I. Netoff, and D. A. Taylor (2008) Perfusion-decellularized matrix: Using nature's platform to engineer a bioartificial heart. *Nat. Med.* 14: 213-221.
64. Yoo, J. J., J. Meng, F. Oberpenning, and A. Atala (1998) Bladder augmentation using allogenic bladder submucosa seeded with cells. *Urol.* 51: 221-225.
65. Cho, S. W., S. H. Lim, I. K. Kim, Y. S. Hong, S. S. Kim, K. J. Yoo, H. Y. Park, Y. Jang, B. C. Chang, C. Y. Choi, K. C. Hwang, and B. S. Kim (2005) Small-diameter blood vessels engineered with bone marrow-derived cells. *Ann. Surg.* 241: 506-515.
66. Cho, S. W., J. E. Lim, H. S. Chu, H. J. Hyun, C. Y. Choi, K. C. Hwang, K. J. Yoo, D. I. Kim, and B. S. Kim (2006) Enhancement of *in vivo* endothelialization of tissue-engineered vascular grafts by granulocyte colony-stimulating factor. *J. Biomed. Mater. Res. A* 76: 252-263.
67. Cho, S.W., I. K. Kim, J. M. Kang, K. W. Song, H. S. Kim, C. H. Park, K. J. Yoo, and B. S. Kim (2009) Evidence for *in vivo* growth potential and vascular remodeling of tissue-engineered artery. *Tissue Eng. Part A* 15: 901-912.
68. Zheng, X. F., S. B. Lu, W. G. Zhang, S. Y. Liu, J. X. Huang, and Q. Y. Guo (2011) Mesenchymal stem cells on a decellularized cartilage matrix for cartilage tissue engineering. *Biotechnol. Bioproc. Eng.* 16: 593-602.
69. Macchiarini, P., P. Jungebluth, T. Go, M. A. Asnaghi, L. E. Rees, T. A. Cogan, A. Dodson, J. Martorell, S. Bellini, P. P. Parnigotto, S. C. Dickinson, A. P. Hollander, S. Mantero, M. T. Conconi, and M. A. Birchall (2008) Clinical transplantation of a tissue-engineered airway. *Lancet* 372: 2023-2030.
70. Uygun, B. E., A. Soto-Gutierrez, H. Yagi, M. L. Izamis, M. A. Guzzardi, C. Shulman, J. Milwid, N. Kobayashi, A. Tilles, F. Berthiaume, M. Hertl, Y. Nahmias, M. L. Yarmush, and K. Uygun (2010) Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. *Nat. Med*. 16: 814-820.
71. Baptista, P. M., M. M. Siddiqui, G. Lozier, S. R. Rodriguez, A. Atala, and S. Soker (2011) The use of whole organ decellularization for the generation of a vascularized liver organoid. *Hepatol.* 53: 604-617.
72. Soto-Gutierrez, A., L. Zhang, C. Medberry, K. Fukumitsu, D. Faulk, H. Jiang, J. Reing, R. Gramignoli, J. Komori, M. Ross, M. Nagaya, E. Lagasse, D. Stolz, S. C. Storm, I. J. Fox, and S. F. Badylak (2011) A whole-organ regenerative medicine approach for liver replacement. *Tissue Eng. Part C Methods* 17: 677-686.
73. Lang, R., M. M. Stern, L. Smith, Y. Liu, S. Bharadwaj, G. Liu, P. M. Baptista, C. R. Bergman, S. Soker, J. J. Yoo, A. Atala, and Y. Zhang (2011) Three-dimensional culture of hepatocytes on porcine liver tissue-derived extracellular matrix. *Biomat.* 32: 7042-7052.
74. Wang, Y., C. B. Cui, M. Yamauchi, P. Miguez, M. Roach, R. Malavarca, M. J. Costello, V. Cardinale, E. Wauthier, C. Barbier, D. A. Gerber, D. Alvaro, and L. M. Reid (2011) Lineage restriction of human hepatic stem cells to mature fates is made efficient by tissue-specific biomatrix scaffolds. *Hepatol.* 53: 293-305.
75. Underhill, G. H., A. A. Chen, D. R. Albrecht, and S. N. Bhatia (2007) Assessment of hepatocellular function within PEG hydrogels. *Biomat.* 28: 256-270.
76. Itle, L. J., W. G. Koh, and M. V. Pishko (2005) Hepatocyte viability and protein expression within hydrogel microstructures. *Biotechnol. Prog.* 21: 926-932.
77. Chen, A. A., D. K.Thomas, L. L. Ong, R. E. Schwartz, T. R. Golub, and S. N. Bhatia (2011) Humanized mice with ectopic artificial liver tissues. *Proc. Natl. Acad. Sci. USA* 108: 11842-11847.
78. Wang, S., D. Nagrath, P. C. Chen, F. Berthiaume, and M. L. Yarmush (2008) Three-dimensional primary hepatocyte culture in synthetic self-assembling peptide hydrogel. *Tissue Eng. Part A* 14: 227-236.
79. Genové, E., S. Schmitmeier, A. Sala, S. Borrós, A. Bader, L. G. Griffith, and C. E. Semino (2009) Functionalized self-assembling peptide hydrogel enhance maintenance of hepatocyte activity *in vitro*. *J. Cell. Mol. Med.* 13: 3387-3397.
80. Kedem, A., A. Perets, I. Gamlieli-Bonshtein, M. Dvir-Ginzberg, S. Mizrahi, and S. Cohen (2005) Vascular endothelial growth factor-releasing scaffolds enhance vascularization and engraftment of hepatocytes transplanted on liver lobes. *Tissue Eng.* 11: 715-722.
81. Jeon, O., S. H. Ryu, J. H. Chung, and B. S. Kim(2005) Control of basic fibroblast growth factor release from fibrin gel with heparin and concentrations of fibrinogen and thrombin. *J. Control. Release* 105: 249-259.
82. Jeon, O., S. W. Kang, H. W. Lim, J. H. Chung, and B. S. Kim (2006) Long-term and zero-order release of basic fibroblast growth factor from heparin-conjugated poly(L-lactide-co-glycolide) nanospheres and fibrin gel. *Biomat.* 27: 1598-1607.
83. Cho, S. W., S. J. Gwak, S. W. Kang, S. H. Bhang, K. W. Song, Y.

S. Yang, C. Y. Choi, and B. S. Kim (2006) Enhancement of angiogenic efficacy of human cord blood cell transplantation. *Tissue Eng.* 12: 1651-1661.
84. Bhang, S. H., S. W. Cho, J. M. Lim, J. M. Kang, T. J. Lee, H. S. Yang, Y. S. Song, M. H. Park, H. S. Kim, K. J. Yoo, Y. Jang, R. Langer, D. G. Anderson, and B. S. Kim (2009) Locally delivered growth factor enhances the angiogenic efficacy of adipose-derived stromal cells transplanted to ischemic limbs. *Stem Cells* 27: 1976-1986.
85. Cho, S. W., I. K. Kim, S. H. Bhang, B. Joung, Y. J. Kim, K. J. Yoo, Y. S. Yang, C. Y. Choi, and B. S. Kim (2007) Combined therapy with human cord blood cell transplantation and basic fibroblast growth factor delivery for treatment of myocardial infarction. *Eur. J. Heart Fail.* 9: 974-985.
86. Hou, Y. T., H. Ljima, T. Takei, and K. Kawakami (2011) Growth factor/heparin-immobilized collagen gel system enhances viability of transplanted hepatocytes and induces angiogenesis. *J. Biosci. Bioeng.* 112: 265-272.
87. Lee, H., R. A. Cusick, F. Browne, T. Ho Kim, P. X. Ma, H. Utsunomiya, R. Langer, and J. P. Vacanti (2002) Local delivery of basic fibroblast growth factor increases both angiogenesis and engraftment of hepatocytes in tissue-engineered polymer devices. *Transplantation* 73: 1589-1593.
88. Hou, Y. T., H. Ljima, S. Matsumoto, T. Kubo, T. Takei, S. Sakai, and K. Kawakami (2010) Effect of a hepatocyte growth factor/heparin-immobilized collagen system on albumin synthesis and spheroid formation by hepatocytes. *J. Biosci. Bioeng.* 110: 208-216.
89. Kim, M., J. Y. Lee, C. N. Jones, A. Revzin, and G. Tae (2010) Heparin-based hydrogel as a matrix for encapsulation and cultivation of primary hepatocytes. *Biomat.* 31: 3596-3603.