13

Liver Regeneration and Bioengineering – The Emergence of Whole Organ Scaffolds

Pedro M. Baptista, Dipen Vyas and Shay Soker
Wake Forest University Health Sciences, Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, USA

1. Introduction

Every year an estimated two million people die of advanced liver disease. The World Health Organization estimates that over six hundred and fifty million people worldwide are affected by some form of liver disease, including thirty million Americans. On a worldwide base, the bleak cenario of one to two million deaths are accounted to liver related diseases annually. From all the countries, China has the world's largest population of Hepatitis B patients (approx. 120 million) with five hundred thousand people dying of the liver disease every year(1, 2). In the US alone, there are around five hundred thousand critical episodes of liver problems every year requiring hospitalization with a huge burden to the patients and an enormous cost to the health care system. In the European Union and United States of America alone, over eighty one thousand and twenty six thousand people died of chronic liver disease in 2006, respectively(1, 3). For these patients, liver transplantation is currently the only therapy proven to extend survival for end-stage liver disease, as it is also the only treatment for severe acute liver failure and the some forms of inborn errors of metabolism. However, the waiting list for liver transplants is extensive and many on the list will not receive an organ due to a dramatic shortage of donors or not being eligible(1).

A good example of this is that in 2007 there were nearly seventeen thousand individuals on the US waiting list for a liver transplant. Only 30% of those in need were transplanted. The average waiting time was more than 400 days. The same year, about one thousand and three hundred people died while waiting for a suitable donor with no available medical option for saving their life. Also, for those patients with fulminant hepatic failure, a severe liver disease with 60-90% mortality, depending on the cause, only 10% received a transplant. Nevertheless, liver transplantation still has a relatively high mortality of 30-40% at 5-8 years with 65% of the deaths occurring in the first 6 months. In addition, patients who have undergone transplantation have to use lifelong immunosuppressive therapy, with sometimes severe side effects(4).

The etiologies of end-stage chronic liver disease that lead to transplantation are numerous and ~80% of people in the liver transplant waiting list have as primary diagnosis a cirrhotic liver. Fortunately, some of the causes of the disease are nowadays preventable. A good example is the successful vaccination programs in many countries in the world against Hepatitis B virus that have considerably reduced the incidence of chronic carriers and viral

induced cirrhosis(5). Regrettably, close to 20% of the livers transplanted in the USA and 30% in Europe have a preventable underlying cause, alcoholic liver disease. Also ~45% of deaths due to liver cirrhosis in the USA are related with alcohol abuse(1, 3, 4). Patients with pathologies like hepatic cancer, congenital malformations and metabolic diseases, and acute hepatic necrosis compose the remaining percentage of the list.

2. Transplantation

The success of liver transplantation has resulted in a progressively increasing demand for such treatment. However, as mentioned above, at the same time the availability of donor organs has diminished, resulting in the number of potential recipients for liver transplantation far exceeding organ supply. Given this, several strategies have been explored in the last decade or so with the aim to increase access to liver transplantation. These include obtaining organs from non-heart-beating donors and live donors, and splitting and using livers from expanded donor criteria. Also, the introduction of the Model for End-Stage Liver Disease (MELD) system implemented February 27 of 2002 in the United States helped Organ Procurement Organizations to prioritize patients waiting for a liver transplant. The MELD score is a numerical scale used for adult liver transplant candidates that ranges between 6 (less ill) and 40 (gravely ill). The individual score determines how urgently a patient needs a liver transplant within the next 3 months. The number is calculated using the most recent laboratory tests – table 1(6).

Bilirubin	which measures how effectively the liver excretes bile
INR	formally known as the prothrombin time, measures the liver's ability to make blood clotting factors
Creatinine	which measures kidney function. Impaired kidney function is often associated with severe liver disease

Table 1. Laboratory values used in the MELD score calculation

The MELD score is then distributed in 4 levels according with the severety of the disease. Less than or equal to 10, 11-18, 19-24 and greater than or equal to 25, being the last the level that includes the most severe patients. Nevertheless, MELD score is not the only factor used for organ allocation to a patient.

In general, for organ distribution a donor is matched to a potential recipient on the basis of several factors: ABO blood type, body size, degree of medical urgency and MELD score. Organ Procurement Organizations (e.g. OPTN/UNOS, etc) uses a computerized point system to distribute organs in a fair manner. Recipients are chosen primarily on the basis of medical urgency within each ABO blood group. Waiting time is only a factor when patients have the same MELD score.

Nevertheless, there are four Special Case Exceptions that will be assigned a higher MELD score than that assigned by the patient's laboratory test results:

- Hepatocellular Carcinoma
- Hepatopulmonary Syndrome
- Familial Amyloidosis
- Primary Oxaluria

In addition to the previously mentioned four special case exceptions, a transplant center can apply for a MELD exception for a patient whose medical urgency is not reflected by the MELD score(6).

The implementation of more fair and efficient allocation systems, improvement in the immunosupressive regimens, and the increase of living donation have all helped to increase overall patient survival and graft survival in the past decade in the United States. The number of livers transplanted also increased to a all time high in 2006, with a marked decrease on the waiting time for liver transplantation after MELD implementation, especially for the sickest patients.

An example of the impact of these improvements is the increase of 6% (86% in 2007) and 16% (87% in 2007) of the unadjusted 1-year graft survival for deceased donor and living donor liver recipients between 1998 and 2007, respectively. These accounts also for an improvement of 3% (89% in 2007) and 11% (91% in 2007) of the unadjusted 1-year patient survival for deceased donor and living donor liver recipients for the same period, respectively(7). However, these numbers decrease significantly for the 5-year patient survival. In 2007 it was 74% and 79% for deceased donor and living donor liver recipients, respectively. These numbers decrease even further for the 10-year patient survival, where in 2007 we had 61% and 71% patient survival for deceased donor and living donor liver recipients, respectively. One important note is that patient survival was higher than graft survival ~5%, because of the opportunity for repeat liver transplantation in the event of graft failure(8).

These numbers highlight the need for novel therapies that can increase patient survival, as well as lower costs to the health care systems. Tolerance research and its clinical induction is a good example of this. The identification of molecular signatures in tolerant patients in whom immunosuppression could be stopped, and induction of tolerance, through lymphocyte depletion or T lymphocyte co-stimulation blockade, are the most advanced approaches to reduce complications of immunosuppression(9).

3. Bioartificil liver devices

In the past few decades, due to scarcity of donors, extracorporeal liver support devices have been developed to support the failing liver resulting from different complications. These devices were created initially for the management of patients waiting for a suitable donor for orthotopic liver transplantation. Recent advances in the design of these devices allow now utilizing them in the recovery of the native liver from an acute injury. Thus, these devices can either bridge the patients to liver transplantation or can fully avoid the need for it (10). Liver support devices can be broadly classified into two classes: artificial liver (AL) devices and bioartificial (BAL) devices. The artificial support devices are designed to detoxify the blood or plasma via different methods like hemodialysis, hemofiltration, hemodiafiltration, hemadsorption, plasmapheresis, plasma fractionation and albumin dialysis (10, 11). Bioartificial support devices are targeted towards providing essential metabolic and synthetic functions of liver along with removal of toxins. BAL devices generally utilize primary hepatocytes or hepatoma cell lines incorporated into a bioreactor system to perform the essential liver functions (12). Here, we will discuss the operating principles of several artificial and bioartificial support systems which have been or are currently used in clinical trials.

3.1 Artificial liver devices

Various metabolic functions of the liver are severely impaired during acute or chronic liver failure which leads to accumulation of lethal toxins in the body. ALs were developed as support devices which can efficiently remove these toxins from blood or plasma by using membrane filtration and/or adsorbents. Liver Dialysis device, Molecular Adsorbent Recirculating System (MARS) and Prometheus are the most widely used artificial support systems.

In liver dialysis device the patient's blood is drawn from a central vein and passed through a low-to-medium permeability membrane and at the same time a suspension of powdered activated charcoal and cation exchange resin is pumped through the dialysate side of the dialyser. This result in removal of toxins from the blood as the toxins are adsorbed based on their binding affinities (10, 13). The MARS uses an albumin-impermeable membrane (50 kDa cutoff) which separates the high-flux albumin coated dialyser from albumin filled dialysate. The toxins bound to the albumin are dissolved from the patient's albumin and they pass through the membrane and ultimately bind to the albumin solution in dialysate side. This albumin is then recycled by dialysis or adsorption through charcoal and resin-binding columns (14, 15). Prometheus primarily uses fractional plasma separation and adsorption techniques for removal of toxins. It uses an albumin permeable membrane (250 kDa cutoff) in contrast to MARS. The albumin bound toxins passes through the membrane and passes through special adsorbers which directly remove the toxins from the plasma and delivering the free albumin back to the patient (13, 14).

Liver Dialysis, MARS and Prometheus have been widely used in clinical trials across Europe and Asia, and have showed some benefits to the patients but no major outcome benefits as far as patient survival is concerned.

3.2 Bioartificial liver devices

Although ALs have been shown to provide temporary support to the patients with acute liver failure by detoxifying the blood or plasma, they have major limitations in replacing synthetic and metabolic functions of liver (12). Thus, attempts have been made to develop bioartificial liver (BAL) systems, which can provide both metabolic and synthetic hepatic functions and its detoxification. BALs incorporate primary hepatocytes or hepatoma cell lines as a biological component and hollow fiber or porous matrix membranes on which the functional hepatocytes are coated (12, 16). Many cell types from various sources have been investigated for providing bioartificial liver support. Primary human hepatocytes have been widely studied as an ideal cell source due to their biocompatibility but they are not readily available and their proliferative capacity *in vitro* is limited (13, 16). Animal cell source such as porcine primary hepatocytes are being investigated due to ease of availability and their ability to maintain metabolic functions similar to human hepatocytes. However, concerns regarding immunological reaction to the animal proteins and transmission of disease exist (17). Nonetheless, porcine hepatocytes remain a popular choice as a hepatocyte source in various BAL systems.

A bioreactor is a vital component of BALs and it has a major influence on the efficacy of these systems. In order to be used in BAL systems, the bioreactor should be able to provide a

suitable environment for hepatic cells to thrive and remain functional along with an adequate interface between blood and hepatocytes for mass transport (18). The bioreactors should also have a potential for scale up and flexibility as BALs may be required to be customized to the patient's needs. Thus, there is an inevitable need for structural optimization and modifications of the bioreactors even though there have been recent advances in this technology. It should be highlighted that no bioreactor is currently approved for patient use, although some have been used in clinical trials (19). Here is a list of BAL devices currently under clinical investigation:

1. Extracorporeal Liver Assist Device (ELAD)
2. HepatAssist
3. Bioartificial Liver Support System (BLSS)
4. The Academic Medical Center – Bioartificial Liver (AMC-BAL)
5. Modular Extracorporeal Liver Support device (MELS)

So far over 200 patients have been treated with HepatAssist and over 40 patients treated with ELAD, making them the most common BALs used for treatment (15, 20). ELAD is the only BAL system which uses human hepatocyte cell line. The other BAL systems listed above use porcine hepatocytes as a cell source. ELAD uses immortalized C3A cell line derived from human hepatoma cell line HepG2 (21). The cells are located in the extracapillary space of hollow fibre cartridges (200 gram total cells in four cartridges). The membrane is impermeable to immunoglobulins, blood cells and C3A cells. The blood flows through the lumen of cartridges as the ultrafiltrated plasma from the membrane comes in direct contact with hepatocytes (12). HepatAssist incorporates approximately 5-7 billion cryopreserved porcine hepatocytes attached to microcarriers and loaded onto a hollow fibre. The separated plasma passes through a charcoal column and oxygenator prior to entering the hollow fibres in the bioreactor. An upgraded version of HepatAssist contains 15-20 billion porcine hepatocytes. The membrane pores are 0.15μm in size which prevents a physical contact between human cells and porcine hepatocytes (10, 20). Currently, none of the BALs have been approved by FDA for clinical use. All of the above listed BALs are undergoing several clinical trials in USA and Europe.

3.3 Future of liver support devices

Recent developments in artificial and bioartificial devices have shown a promising path towards the management of patients with acute liver failure. However, considerable technical challenges and regulatory issues remain to be tackled in order to efficiently utilize these devices in the clinic. ALs have demonstrated the ease of use and cost effectiveness along with proving to improve biochemical parameters and clinical symptoms by detoxifying the blood/plasma, but it has a major limitation of not replacing critical metabolic and synthetic functions of liver. In order to ameliorate liver injury and subsequently prevent the lethal effects of loss of liver function on other critical organs, liver function needs to be performed by the extracorporeal support devices while the patient awaits the transplantation. BALs have been developed in recent years which utilize hepatocytes in a bioreactor to carry out the metabolic and synthetic functions of liver. For these reasons, BALs hold a promising future as they have shown potential by efficiently treating several patients across different clinical trials. Several challenges exist in BAL technology including the debate on ideal cell source, requirement of large number of cells,

maintainance of the functional hepatocytes for longer period of time in a bioreactor, complexity of the design and high cost. These challenges have delayed the entry of BAL systems in the clinic. Nonetheless, plenty of optimized designs of liver support devices are under development and undergoing clinical trials which is a sign of optimism in this area of critical care and management.

4. Cell therapies

Hepatocyte transplantation is certainly in the forefront of new therapeutic strategies. The first successful hepatocyte transplantation into a patient was carried out in June 1992 to a French Canadian woman with familial hypercholesterolaemia. After *ex vivo* transduction with a retrovirus encoding for the human LDL receptor, the patient's hepatocytes were infused through the inferior mesenteric vein into the liver. LDL and HDL levels improved throughout the next 18 months and transgene expression was detected in a liver biopsy(22). Following this first success, other patients followed through. However, not all the patients treated had a clear benefit from the procedure(23). Since then, several other metabolic diseases have been treated with hepatocyte transplantation with different degrees of success(24-28). It has also been used as a support treatment to acute(29-31) and chronic liver diseases(30-33) in bridging severely ill patients to orthotopic liver transplantation (OLT). Low efficacy and lack of long-term therapeutic effect have been common in all these procedures. These failures could be explained by the relatively small number of hepatocytes that engraft in the recipient liver due to quality, quantity and possibly immunosuppresion protocols(34). However, transplantation of a number of hepatocytes corresponding to 1-5% of the total liver mass has been able to show a positive impact in transplanted patients, even if for a limited period of time(34).

Due to the shortage of available human hepatocytes for transplantation, other cell sources have been used. Specifically, bone marrow derived mesenchymal stem cells(35), hematopoietic stem cells(36, 37) and fetal liver progenitor/stem cells(38) have shown to improve, to a certain extent, the condition of cirrhotic patients. The latter cell type holds an enormous potential for cell/regenerative medicine therapies due to their high expansion capabilities and differentiation into hepatocytes and biliary epithelium(39).

Recent data suggests that human embrionic (hES) and induced pluripotent (iPS) stem cells hold great promise to regenerative applications in every medical field. Specifically for the liver, several studies have established the required pathways to differentiate a hES or iPS into a hepatic fate by using defined soluble growth factor signals that mimic embryonic development(40, 41). These cells, once transplanted into rodent livers were able to engraft and express several normal hepatic functions(42). However, more extensive characterization, as well as further safety evaluation, are needed to determine wether these cells will fully function as primary adult hepatocytes.

5. Liver bioengineering

Tissue engineering is one of the most promising fields in regenerative medicine. As described in 1993 by Robert Langer and Joseph Vacanti it is the conjugation of biomaterials (synthetic or naturally derived) with cells, in order to generate tissue constructs that can be implanted into patients to substitute a lost function, maintain or gain new functions(43). The

current paradigm is suitable for the engineering of thin constructs like the bladder, skin or blood vessels. Although, in the specific case of the liver, the 3D architecture and dense cellular mass requires novel tissue engineering approaches and the development of vascularized biomaterials, in order to support thick tissue masses and be readily transplantable. Additionally to the vascular support for large tissue masses, hepatocyte function maintenance represents the ultimate aim in any organ engineering or regenerative medicine strategy for liver disease.

Hepatocytes are known to be attachment-dependent cells and lose rather quickly their specific functions without optimal media- and ECM- composition and cell-cell contacts. Also, function and differentiation of liver cells are influenced by the 3D organ architecture(44).

In the last two decades innumerous strategies for the culture of adult hepatocytes in combination with several types of 3D, highly porous polymeric matrices have been attempted(45-49). However, in the absence of vasculature, restriction in cell growth and function is common due to the limitations in nutrient and oxygen diffusion. Some of these problems are being now partially overcome with the development of bioreactors that provide continuous perfusion of culture media and gases allowing a 3D culture configuration and hepatocyte function maintenance(50-52).

The tissue engineering concept has several advantages over the injection of cell suspensions into solid organs. The matrices provide sufficient volume for the transplantation of an adequate cell mass up to whole-organ equivalents[45]. Transplantation efficiency could readily be improved by optimizing the microarchitecture and composition of the matrices as well as by attaching growth factors and extracellular matrix molecules to the polymeric scaffold, helping to recreate the hepatic microenvironment(44). The use of naturally derived matrices has also proved to be very helpful in hepatocyte culture(47). These matrices, besides preserving some of the microarchitecture features of the tissues that they are derived from, also retain bioactive signals (e.g., cell-adhesion peptides and growth factors) required for the retention of tissue-specific gene expression(53, 54). Additionally, cell transplantation into polymeric matrices is, in contrast to cell injection into tissues and organs, a reversible procedure since the cell-matrix-constructs may be removed if necessary.

Finally, heterotopic hepatocyte transplantation in matrices has already been demonstrated in long-term studies(55, 56). Nonetheless, initial engraftment rates are suboptimal. One of the reasons for this is the absolute requirement of the transplanted hepatocytes for hepatotrophic factors that the liver constantly receives through its portal circulation(57). Thus, the development of a tissue engineered liver construct capable of being orthotopically transplanted is essential.

Apart from cellular therapies, other early developments of experimental approaches are not showing results that will indicate clinical translation in the next few years. However, two experimental approaches that show higher level of maturity may have the potential for succesful clinical translation. The first experimental approach is the “cell sheet” technology developed by Okano *et al*. in Japan(58). Its simple configuration and fabrication allows for the stacking of up to four hepatocyte cell sheets that can readily engraft and provide a defined metabolic relief to the recipient(59). This technology has already been applied successfully to one patient with heart failure. Other technology that shows great promise is

tissue and organ decellularization. Our lab and others have been able to generate several decellularized scaffolds for tissue engineering applications like tissue engineering of urethra(60), heart valves(61), blood vessel(62). More recentely, Ott *et al*. developed a novel method of perfusion decellularization that is able to generate whole organ scaffolds. The use of this method allowed the decellularization of a whole heart that was later repopulated with neonatal rat cardiomyocytes. This bioengineered heart was able to contract up to 2% of the normal contractile function(63). This approach may have a tremendous potential for the field of organ bioengineering. We have recently used a similar perfusion decellularization technique to liver, pancreas, intestine and kidney generating decellularized organ scaffolds for organ bioengineering(64, 65). These bioscaffolds preserve their tissue microarchitecture and an intact vascular network that can be readily used as a route for recellularization by perfusion of culture medium with different cell populations. In an analogous fashion, Uygun *et al*. decellularized rat livers and repopulated them with rat primary hepatocytes, showing promising hepatic function and the ability of heterotopicaly transplant these bioengineered livers into animals for up to eight hours(66). Baptista *et al.* were able to take this a step further by using human primary liver progenitor/stem and endothelial cells to bioengineer a vascularized liver. These bioengineered livers displayed some of the functions of a native human liver (albumin and urea secretion, drug metabolism enzyme expression, etc), exhibiting also an endothelialized vascular network that prevented platelet adhesion and aggregation, critical for blood vessel patency after transplantation(65). Nonetheless, it is difficult to predict the outcome and the real translational value of this technology in the present days, but the potential is certainly vast. Translation of it into the bioengineering of human size livers might help mitigate the endless hurdle of organ shortage for transplantation.

6. References

[1] CDC. Centers for Disease Control and Prevention Database. In; 2007.
[2] WHO. World Health Organization - Global Burden of Disease: 2004 update (2008). In: WHO publications; 2008.
[3] Eurostat. Eurostat's Harmonised Regional Statistical Database. In; 2007.
[4] OPTN. Transplant Database. In; 2011.
[5] Kao JH, Chen DS. Global control of hepatitis B virus infection. Lancet Infect Dis 2002;2:395-403.
[6] OPTN. MELD score. In; 2011.
[7] Wolfe RA, Merion RM, Roys EC, Port FK. Trends in organ donation and transplantation in the United States, 1998-2007. Am J Transplant 2009;9:869-878.
[8] Thuluvath PJ, Guidinger MK, Fung JJ, Johnson LB, Rayhill SC, Pelletier SJ. Liver transplantation in the United States, 1999-2008. Am J Transplant;10:1003-1019.
[9] Turka LA, Wood K, Bluestone JA. Bringing transplantation tolerance into the clinic: lessons from the ITN and RISET for the Establishment of Tolerance consortia. Curr Opin Organ Transplant;15:441-448.
[10] Carpentier B, Gautier A, Legallais C. Artificial and bioartificial liver devices: present and future. Gut 2009;58:1690-1702.
[11] Phua J, Lee KH. Liver support devices. Curr Opin Crit Care 2008;14:208-215.
[12] Park JK, Lee DH. Bioartificial liver systems: current status and future perspective. J Biosci Bioeng 2005;99:311-319.
[13] Pless G. Artificial and bioartificial liver support. Organogenesis 2007;3:20-24.

[14] Rifai K. Extracorporeal albumin dialysis. Hepatol Res 2008;38:S41-S45.
[15] Brophy CM, Nyberg SL. Extracorporeal treatment of acute liver failure. Hepatol Res 2008;38:S34-S40.
[16] Cao S, Esquivel CO, Keeffe EB. New approaches to supporting the failing liver. Annu Rev Med 1998;49:85-94.
[17] Stange J, Mitzner S. Cell sources for bioartificial liver support. Int J Artif Organs 1996;19:14-17.
[18] Tilles AW, Berthiaume F, Yarmush ML, Tompkins RG, Toner M. Bioengineering of liver assist devices. J Hepatobiliary Pancreat Surg 2002;9:686-696.
[19] Yu CB, Pan XP, Li LJ. Progress in bioreactors of bioartificial livers. Hepatobiliary Pancreat Dis Int 2009;8:134-140.
[20] McKenzie TJ, Lillegard JB, Nyberg SL. Artificial and bioartificial liver support. Semin Liver Dis 2008;28:210-217.
[21] Adham M. Extracorporeal liver support: waiting for the deciding vote. ASAIO J 2003;49:621-632.
[22] Grossman M, Raper SE, Kozarsky K, Stein EA, Engelhardt JF, Muller D, Lupien PJ, et al. Successful ex vivo gene therapy directed to liver in a patient with familial hypercholesterolaemia. Nat Genet 1994;6:335-341.
[23] Grossman M, Rader DJ, Muller DW, Kolansky DM, Kozarsky K, Clark BJ, 3rd, Stein EA, et al. A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. Nat Med 1995;1:1148-1154.
[24] Fox IJ, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI, Dorko K, et al. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl J Med 1998;338:1422-1426.
[25] Horslen SP, McCowan TC, Goertzen TC, Warkentin PI, Cai HB, Strom SC, Fox IJ. Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. Pediatrics 2003;111:1262-1267.
[26] Ambrosino G, Varotto S, Strom SC, Guariso G, Franchin E, Miotto D, Caenazzo L, et al. Isolated hepatocyte transplantation for Crigler-Najjar syndrome type 1. Cell Transplant 2005;14:151-157.
[27] Muraca M, Gerunda G, Neri D, Vilei MT, Granato A, Feltracco P, Meroni M, et al. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. Lancet 2002;359:317-318.
[28] Sokal EM, Smets F, Bourgois A, Van Maldergem L, Buts JP, Reding R, Bernard Otte J, et al. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. Transplantation 2003;76:735-738.
[29] Strom SC, Fisher RA, Thompson MT, Sanyal AJ, Cole PE, Ham JM, Posner MP. Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. Transplantation 1997;63:559-569.
[30] Strom SC, Chowdhury JR, Fox IJ. Hepatocyte transplantation for the treatment of human disease. Semin Liver Dis 1999;19:39-48.
[31] Strom SC, Fisher RA, Rubinstein WS, Barranger JA, Towbin RB, Charron M, Mieles L, et al. Transplantation of human hepatocytes. Transplant Proc 1997;29:2103-2106.

[32] Combs C, Brunt EM, Solomon H, Bacon BR, Brantly M, Di Bisceglie AM. Rapid development of hepatic alpha1-antitrypsin globules after liver transplantation for chronic hepatitis C. Gastroenterology 1997;112:1372-1375.
[33] Mito M, Kusano M, Kawaura Y. Hepatocyte transplantation in man. Transplant Proc 1992;24:3052-3053.
[34] Fisher RA, Strom SC. Human hepatocyte transplantation: worldwide results. Transplantation 2006;82:441-449.
[35] Kharaziha P, Hellstrom PM, Noorinayer B, Farzaneh F, Aghajani K, Jafari F, Telkabadi M, et al. Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial. Eur J Gastroenterol Hepatol 2009;21:1199-1205.
[36] Salama H, Zekri AR, Zern M, Bahnassy A, Loutfy S, Shalaby S, Vigen C, et al. Autologous hematopoietic stem cell transplantation in 48 patients with end-stage chronic liver diseases. Cell Transplant 2010.
[37] Zacharoulis D, Milicevic MN, Helmy S, Jiao LR, Levicar N, Tait P, Scott M, et al. Autologous infusion of expanded mobilized adult bone marrow-derived CD34+ cells into patients with alcoholic liver cirrhosis. Am J Gastroenterol 2008;103:1952-1958.
[38] Khan AA, Shaik MV, Parveen N, Rajendraprasad A, Aleem MA, Habeeb MA, Srinivas G, et al. Human fetal liver derived stem cell transplantation as supportive modality in the\ management of end stage decompensated liver cirrhosis. Cell Transplantation 2010.
[39] Schmelzer E, Zhang L, Bruce A, Wauthier E, Ludlow J, Yao HL, Moss N, et al. Human hepatic stem cells from fetal and postnatal donors. J Exp Med 2007;204:1973-1987.
[40] Gouon-Evans V, Boussemart L, Gadue P, Nierhoff D, Koehler CI, Kubo A, Shafritz DA, et al. BMP-4 is required for hepatic specification of mouse embryonic stem cell-derived definitive endoderm. Nat Biotechnol 2006;24:1402-1411.
[41] Gadue P, Huber TL, Paddison PJ, Keller GM. Wnt and TGF-beta signaling are required for the induction of an in vitro model of primitive streak formation using embryonic stem cells. Proc Natl Acad Sci U S A 2006;103:16806-16811.
[42] Basma H, Soto-Gutierrez A, Yannam GR, Liu L, Ito R, Yamamoto T, Ellis E, et al. Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. Gastroenterology 2009;136:990-999.
[43] Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920-926.
[44] Mooney D, Hansen L, Vacanti J, Langer R, Farmer S, Ingber D. Switching from differentiation to growth in hepatocytes: control by extracellular matrix. J Cell Physiol 1992;151:497-505.
[45] Fiegel HC, Kaufmann PM, Bruns H, Kluth D, Horch RE, Vacanti JP, Kneser U. Hepatic tissue engineering: from transplantation to customized cell-based liver directed therapies from the laboratory. J Cell Mol Med 2008;12:56-66.
[46] Kim SS, Sundback CA, Kaihara S, Benvenuto MS, Kim BS, Mooney DJ, Vacanti JP. Dynamic seeding and in vitro culture of hepatocytes in a flow perfusion system. Tissue Eng 2000;6:39-44.
[47] Lin P, Chan WC, Badylak SF, Bhatia SN. Assessing porcine liver-derived biomatrix for hepatic tissue engineering. Tissue Eng 2004;10:1046-1053.

[48] Linke K, Schanz J, Hansmann J, Walles T, Brunner H, Mertsching H. Engineered liver-like tissue on a capillarized matrix for applied research. Tissue Eng 2007;13:2699-2707.

[49] Tong JZ, Bernard O, Alvarez F. Long-term culture of rat liver cell spheroids in hormonally defined media. Exp Cell Res 1990;189:87-92.

[50] Gerlach J, Unger J, Hole O, Encke J, Muller C, Neuhaus P. [Bioreactor for long-term maintenance of differentiated hepatic cell functions]. ALTEX 1994;11:207-215.

[51] Torok E, Pollok JM, Ma PX, Kaufmann PM, Dandri M, Petersen J, Burda MR, et al. Optimization of hepatocyte spheroid formation for hepatic tissue engineering on three-dimensional biodegradable polymer within a flow bioreactor prior to implantation. Cells Tissues Organs 2001;169:34-41.

[52] Torok E, Vogel C, Lutgehetmann M, Ma PX, Dandri M, Petersen J, Burda MR, et al. Morphological and functional analysis of rat hepatocyte spheroids generated on poly(L-lactic acid) polymer in a pulsatile flow bioreactor. Tissue Eng 2006;12:1881-1890.

[53] Kim BS, Baez CE, Atala A. Biomaterials for tissue engineering. World J Urol 2000;18:2-9.

[54] Voytik-Harbin SL, Brightman AO, Kraine MR, Waisner B, Badylak SF. Identification of extractable growth factors from small intestinal submucosa. J Cell Biochem 1997;67:478-491.

[55] Kaufmann PM, Kneser U, Fiegel HC, Kluth D, Herbst H, Rogiers X. Long-term hepatocyte transplantation using three-dimensional matrices. Transplant Proc 1999;31:1928-1929.

[56] Johnson LB, Aiken J, Mooney D, Schloo BL, Griffith-Cima L, Langer R, Vacanti JP. The mesentery as a laminated vascular bed for hepatocyte transplantation. Cell Transplant 1994;3:273-281.

[57] Starzl TE, Francavilla A, Halgrimson CG, Francavilla FR, Porter KA, Brown TH, Putnam CW. The origin, hormonal nature, and action of hepatotrophic substances in portal venous blood. Surg Gynecol Obstet 1973;137:179-199.

[58] Yang J, Yamato M, Shimizu T, Sekine H, Ohashi K, Kanzaki M, Ohki T, et al. Reconstruction of functional tissues with cell sheet engineering. Biomaterials 2007;28:5033-5043.

[59] Ohashi K, Yokoyama T, Yamato M, Kuge H, Kanehiro H, Tsutsumi M, Amanuma T, et al. Engineering functional two- and three-dimensional liver systems in vivo using hepatic tissue sheets. Nat Med 2007;13:880-885.

[60] El-Kassaby AW, Retik AB, Yoo JJ, Atala A. Urethral stricture repair with an off-the-shelf collagen matrix. J Urol 2003;169:170-173; discussion 173.

[61] Lee DJ, Steen J, Jordan JE, Kincaid EH, Kon ND, Atala A, Berry J, et al. Endothelialization of heart valve matrix using a computer-assisted pulsatile bioreactor. Tissue Eng Part A 2009;15:807-814.

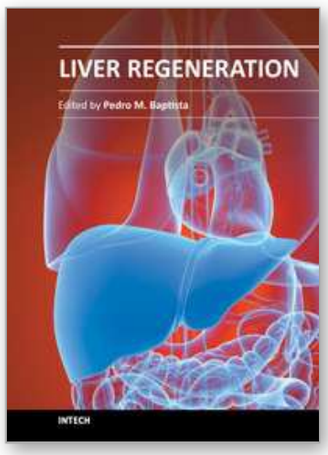
[62] Amiel GE, Komura M, Shapira O, Yoo JJ, Yazdani S, Berry J, Kaushal S, et al. Engineering of blood vessels from acellular collagen matrices coated with human endothelial cells. Tissue Eng 2006;12:2355-2365.

[63] Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med 2008;14:213-221.

[64] Baptista PM, Orlando G, Mirmalek-Sani SH, Siddiqui M, Atala A, Soker S. Whole organ decellularization - a tool for bioscaffold fabrication and organ bioengineering. Conf Proc IEEE Eng Med Biol Soc 2009;2009:6526-6529.

[65] Baptista PM, Siddiqui MM, Lozier G, Rodriguez SR, Atala A, Soker S. The use of whole organ decellularization for the generation of a vascularized liver organoid. Hepatology 2011;53:604-617.

[66] Uygun BE, Soto-Gutierrez A, Yagi H, Izamis ML, Guzzardi MA, Shulman C, Milwid J, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat Med 2010.



Liver Regeneration

Edited by PhD. Pedro Baptista

ISBN 978-953-51-0622-7
Hard cover, 252 pages
Publisher InTech
Published online 16, May, 2012
Published in print edition May, 2012

Doctors and scientists have been aware of the "phenomenom" of liver regeneration since the time of the ancient Greeks, illustrated by the mythic tale of Prometheus' punishment. Nevertheless, true insight into its intricate mechanisms have only become available in the 20th century. Since then, the pathways and mechanisms involved in restoring the liver to its normal function after injury have been resolutely described and characterized, from the hepatic stem/progenitor cell activation and expansion to the more systemic mechanisms involving other tissues and organs like bone-marrow progenitor cell mobilization. This book describes some of the complex mechanisms involved in liver regeneration and provides examples of the most up-to-date strategies used to induce liver regeneration, both in the clinic and in the laboratory. The information presented will hopefully benefit not only professionals in the liver field, but also people in other areas of science (pharmacology, toxicology, etc) that wish to expand their knowledge of the fundamental biology that orchestrates liver injury and regeneration.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Pedro M. Baptista, Dipen Vyas and Shay Soker (2012). Liver Regeneration and Bioengineering - The Emergence of Whole Organ Scaffolds, Liver Regeneration, PhD. Pedro Baptista (Ed.), ISBN: 978-953-51-0622-7, InTech, Available from: http://www.intechopen.com/books/liver-regeneration/liver-bioengineering-the-emergence-of-whole-organ-scaffolds

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen