Tissue Engineering Review Series *J. Cell. Mol. Med. Vol 12, No 1, 2008 pp. 56-66*

Guest Editor: R. E. Horch

Hepatic tissue engineering: from transplantation to customized cell-based liver directed therapies from the laboratory

Henning C. Fiegel [a, *, #], Peter M. Kaufmann [b, #], Helge Bruns [a], Dietrich Kluth [a], Raymund E. Horch [c], Joseph P. Vacanti [d], Ulrich Kneser [c]

[a] *Department of Pediatric Surgery, University of Leipzig, Leipzig, Germany*
[b] *Department of Urology, University of Hannover, Hannover, Germany*
[c] *Department of Plastic and Hand Surgery, University Hospital Erlangen, Erlangen, Germany*
[d] *Department of Pediatric Surgery, Massachusetts General Hospital, Harvard University, Boston, MA, USA*

Received: July 28, 2007; Accepted: October 24, 2007

Abstract

Today, liver transplantation is still the only curative treatment for liver failure due to end-stage liver diseases. Donor organ shortage, high cost and the need of immunosuppressive medications are still the major limitations in the field of liver transplantation. Thus, alternative innovative cell-based liver directed therapies, for example, liver tissue engineering, are under investigation with the aim that in future an artificial liver tissue could be created and be used for the replacement of the liver function in patients. Using cells instead of organs in this setting should permit (*i*) expansion of cells in an *in vitro* phase, (*ii*) genetic or immunological manipulation of cells for transplantation, (*iii*) tissue typing and cryopreservation in a cell bank and (*iv*) the *ex vivo* genetic modification of patient's own cells prior to re-implantation. Function and differentiation of liver cells are influenced by the three-dimensional organ architecture. The use of polymeric matrices permits the three-dimensional formation of a neo tissue and specific stimulation by adequate modification of the matrix surface, which might be essential for appropriate differentiation of transplanted cells. In addition, culturing hepatocytes on three-dimensional matrices permits culture in a flow bioreactor system with increased function and survival of the cultured cells. Based on bioreactor technology, bioartificial liver devices (BAL) are developed for extracorporeal liver support. Although BALs improved clinical and metabolic conditions, increased patient survival rates have not been proven yet. For intracorporeal liver replacement, a concept that combines tissue engineering using three-dimensional, highly porous matrices with cell transplantation could be useful. In such a concept, whole liver mass transplantation, long-term engraftment and function as well as correction of a metabolic defect in animal models could be achieved with a principally reversible procedure. Future studies have to investigate which environmental conditions and transplantation system would be most suitable for the development of artificial functional liver tissue including blood supply for a potential use in a clinical setting.

Keywords: liver cell transplantation • hepatic tissue engineering

#These authors contributed equally.
*Correspondence to: Henning C. FIEGEL,
Dept. of Pediatric Surgery, University of Leipzig,
Liebigstrasse 20A, D-04103 Leipzig, Germany.
Tel.: +49 34 19 72 63 65; +49 34 19 72 68 81;
Fax: +49 341 97 26409.
E-mail: henning.fiegel@medizin.uni-leipzig.de

doi: 10.1111/j.1582-4934.2007.00162.x

Introduction

Today, liver transplantation is an established and successful procedure that represents the only causal and curative therapy for many liver diseases that lead to liver cirrhosis and consecutive liver failure in end-stage disease [1]. Despite its therapeutic potential it remains an unspecific approach that is limited by donor organ shortage [2] and the need for a life-long immunosuppressive therapy with its specific risks [3]. According to data from the UNOS Database 6134 livers have been transplanted in 2006, but in March 2007 16,995 candidates where on the waiting list [4]. This situation makes the search for alternatives to whole organ transplantation an important topic in current transplantation research.

Extracorporeal systems seem to be suitable for acute intervention in cases of acute liver failure (ALF) or intoxication, and may serve as a bridge to liver transplantation or organ recovery [5]. However, the long-term application of this concept is problematic due to technical limitations, high costs and the necessity of an intensive care unit setting.

Many liver diseases with primary intact liver function and organ architecture require only the correction or replacement of a small sector of the complex liver function that may in future be accomplished by gene therapy [6]. The transplantation of a hepatocyte mass equivalent to 10% of the patients' liver would be sufficient to normalize the metabolic situation in many enzyme deficiencies [7]. For this purpose intracorporeal systems based on the transplantation of isolated liver cells are desirable. Cell transplantation has some advantages over organ transplantation: one donor organ could be used for many candidates. Because this is a much lesser invasive technique it is associated with a lower mortality and morbidity. Cell transplantation is also thought to be less immunogenic because transplanted allogenic cells could be immunomodulated prior to implantation or even autologous cells might be utilized following *in vitro* modification. Cell transplantation might be an option for patients with metabolic diseases because the complete organ does not need to be replaced and the deficient metabolic function could be replaced or at least supported by only a small portion of hepatocytes.

Development of cell isolation and primary culture for hepatocytes

Cell-based therapies for liver failure ideally require proliferative cells with the capability to differentiate into different types of liver cells. Over the past 50 years a variety of methods have been developed for the isolation of liver cells. The first isolation techniques resulted in a high percentage of damaged cells because mechanical force was used. Anderson *et al*. used a Ca^{2+}-free solution for perfusion of the liver under pressure [8]. Many of the cells isolated this way were damaged. When in 1967 Howard, Christensen, Gibbs and Pesch [9] introduced the enzymatic method of cell isolation, a milestone in hepatocyte isolation was achieved. Berry and Friend [10] modified this method in 1969 and perfused the rat liver via the portal vein for the first time. They were able to convert almost 50% of the rat's liver into viable, intact hepatocytes. Seglen *et al*. improved this method and used a two-step perfusion [11]. Physiological liver perfusion leads to a high yield of intact liver cells, and most efforts in liver cell isolation since Berry and Friend have been dealing with the optimization of temperature, collagenase and Ca^{2+} concentration [12]. The intra- or extracorporeal physiological liver perfusion still is the state-of-the-art technique used for liver cell isolation. Non-enzymatic methods are also considered as a feasible technique for the isolation of adult hepatocytes [13–15]. Other cell types that can be used for hepatic tissue engineering include hematopoietic stem cells, oval progenitor cells, adult hepatocytes and hepatoblastoma-derived cells. Initial data indicate that mesenchymal stromal cells (MSCs) might also generate hepatic progenitor cells *in vitro* under the appropriate culture conditions [16–18]. The factors that are needed for differentiation include growth factors, cues from other cells and extracellular matrix molecules (ECMs). The special conditions in which MSCs can be transdifferentiated into hepatocytes and other cell types are currently investigated [19–21].

Three-dimensional culture using matrices

Hepatocytes are attachment-dependent cells and lose their liver-specific function without optimal media- and ECM composition and cell-cell contacts. In order to develop potent culture systems for hepatocytes, hepatotrophic stimulation of the cells *in vitro* is necessary [22]. Several stimulatory mechanisms are evaluated in hepatocyte cultures:

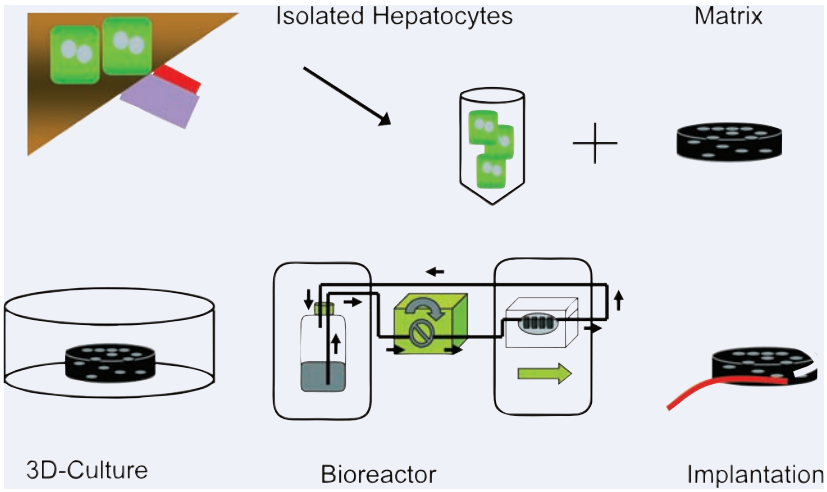

Fig. 1 Scheme of the tissue engineering approach for the liver. Isolated liver cells are seeded on three-dimensional matrices in order to stimulate cell proliferation and hepatocyte-specific differentiation. The cell-seeded matrices can be used for successful three-dimensional culture, culture in a bioreactor system and for transplantation.

(*i*) Coating of culture dishes with isolated ECMs [23, 24], (*ii*) the addition of growth hormones and cytokines to the culture media [25, 26] or (*iii*) co-culture with other cell types [27–30]. In particular the culture configuration has been shown to have a major impact on cellular differentiation: cultures in 'sandwich' configuration could achieve a significant elongation of the culture period, as well as an increase of specific function and cell growth [24]. A variety of novel culture systems for hepatocytes, including hydrogel microspheres, hollow fibres and macroporous polymer scaffolds were developed and shown to promote specific functions, such as albumin secretion or detoxification capacity [31, 32]. Furthermore, initial data suggest a strong positive influence caused by flow in a bioreactor system for hepatocyte culture: Hepatocytes cultured under flow conditions show new tissue formation and high albumin production [33]. This approach seems to be attractive, because it may permit the creation of a functional BAL tissue for transplantation (Fig. 1).

Development of bioreactor systems for liver cells

Currently, adult hepatocytes used for tissue engineering include those derived from immortalized human hepatoblastoma cell lines such as HepG2/C3A. The main advantage is the easy cultivation of large quantities of those cells. When discussing the clinical usage of tumour cell lines the long-term safety is still an important issue, which should be addressed in further studies before clinical use.

For most available BAL systems, porcine hepatocytes are being used. The advantage of these cells is their cheap and easy availability. As long as the porcine hepatocytes are kept outside the patients' circulation there is no danger of immunological reactions. For cell transplantation the possible usage of genetically altered porcine hepatocytes that lack α-galactose is currently discussed [34]. For cell culture different types of bioreactors exist. For BAL devices mostly hollow fibre bioreactors loaded with porcine hepatocytes are used, but there also exist monolayer bioreactors, perfused scaffolds and cell suspensions. The hollow fibre technology, which was developed for kidney dialysis, is an easy technology for BAL. Cells are protected from shear stress and a high number of cells can be cultivated in a small volume because of a big attachment surface [35].

Monolayer cultures using the 'sandwich culture' show good stability of hepatocytes, but the cells are exposed to shear stress when the culture is perfused [36]. These cultures also have a low surface to volume ratio. Perfused scaffolds such as PLGA [37] also show the problem of shear forces; the advantages include ease of scale-up and the positive effects of three-dimensional architecture of cell culture. Suspension cultures show poor cell stability but ease of scale-up and very good transfer between plasma and immobilized hepatocytes [38, 39].

Table 1 Clinical trials of BAL devices

Device	Cell Source	Study Type	Patients Treated	Comments
ELAD	C3A-cells (hepatoma-derived)	Phase I/II	52 [45, 46]	
HepatAssist	Porcine	Phase II/III	171 (86 patients. In control group, 85 received BAL treatment) [47]	Survival advantage in FHF
MELS	Human	Phase I	20 [49, 50]	Patients bridged to transplantation; system based on BELS (which was based on porcine hepatocytes)
AMC-BAL	Porcine	Phase I	8 with acute HF [53]	7 patients bridged to transplantation, 1 recovered
BLSS	Porcine	Phase I/II	4 [82]	Decreased bilirubin, lactate and ammonia levels

BELS, bioartificial extracorporeal liver support.

First clinical application of bioreactors with liver cells

First attempts in artificial liver support were based on charcoal hemoperfusion [40]. Newer systems such as MARS (molecular adsorbents recirculating system, Gambro, Sweden; developed by Stange *et al*. [41]) and FPAD (Fractionated Plasma Separation, Adsorption and Dialysis system, Prometheus, Fresenius Medical Care, Bad Homburg, Germany) eliminate protein-bound bilirubin and bile acids. The effect on mortality seems to be low. BAL systems use viable hepatocytes and are connected to the patient's circulation (Table 1). The concept of artificial liver was developed by Sorrentino in 1956 [42], who proved that liver tissue homogenate could produce urea from ammonia chloride. In 1975 Wolf *et al*. could prove that hepatoma cells placed in the extrafibre space of a hollowfibre cartridge could effectively conjugate bilirubin [43].

For bioreactor cultures various cell sources have been evaluated. Cells used for BAL include human hepatocytes as well as hepatoblastoma-derived cells (C3A cells), but the most used cells are porcine hepatocytes. Porcine hepatocytes are easily cultivated *in vitro* and are available in large quantities but bear the risk of infection (*e.g.* porcine endogenous retrovirus [PERV] or herpes species) and metabolic incompatibility. Human tumour cell lines such as C3A cells can be easily cultivated but have poor liver key functions [44] and a potential tumourigenic ability. Primary human cells meet all the demands of compatibility but usually are not available in appropriate quantities and originate from histologically impaired organs that are not suitable for whole organ transplantation.

The extracorporeal liver assist device (ELAD) uses about 200–400 g of cells of the human hepatoblastoma cell line C3A (derived from HepG2) in modified dialysis-based cartridges. The cells are located in the extracapillary space separated from plasma by a capillary membrane. Prior to entering the bioreactor, the plasma passes an adsorber and a membrane oxygenator. First, clinical applications were performed to demonstrate the safety of the system [45, 46].

The HepatAssist (Circe-Biomed. Inc., Los Angeles, CA, USA) utilizes 5–7 x 10^9 cryopreserved porcine hepatocytes that are placed in the device just before clinical use. The function of the bioreactor is supplemented by a column filled with activated charcoal. The patient's plasma is separated using plasmapheresis, transported through the charcoal column, oxygenated and then sent through the bioreactor. A large randomized, controlled multicenter study with a total of 171 patients (86 in the control

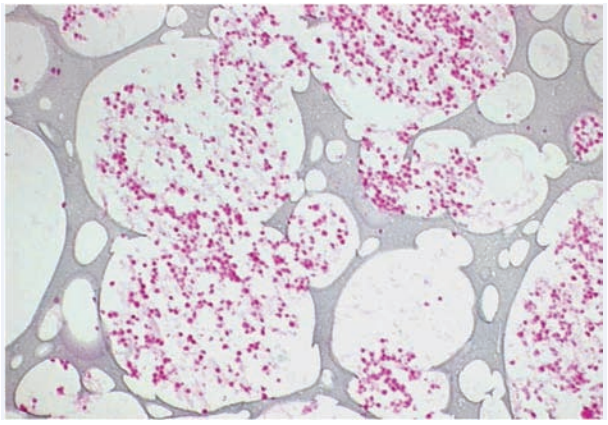

Fig. 2 Rat hepatocytes seeded on a three-dimensional polymeric matrix form a three-dimensional tissue *in vitro* after 3 days in culture.

group and 85 in the bioartificial liver treatment group) was conducted by Demetriou *et al*. [47]. Patients with fulminant/subfulminant hepatic failure and primary nonfunction following liver transplantation were included in the study, demonstrating the safety of the system and an improved 30-day survival in a subgroup. Survival benefit could only be proven for a small group of patients with fulminant liver failure. No PERV could be detected in patients [48].

The MELS (modular extracorporeal liver support) system (Charite, Berlin, Germany) developed by Gerlach *et al*. [5] is based on immobilized hepatocytes [49] in a bioreactor with three independent capillary systems for medium inflow, cell oxygenation and medium outflow. The direct contact of blood cells with the hepatocytes is avoided by a plasmaseparation step and an outflow filtration. A phase I clinical study was performed with the CellModule [50] charged with porcine hepatocytes and 12 patients were treated with human hepatocytes [50]. In 2002 eight patients with ALF could be bridged successfully to transplantation using the MELS system [51]. Another BAL device, the AMC (Amsterdam Medical Centre)-BAL developed by Chamuleau showed significant improvement in hepatic encephalopathy and detoxification in case reports and in one phase I trial [52, 53]. The AMC-BAL uses porcine hepatocytes that are cultivated on a spirally wound polyester fabric. Hollow fibres are used for oxygenation. Preclinical studies suggest that the BLSS (bioartificial liver support system; Excorp Medical, Inc. Minneapolis, Minnesota, USA) impacts the course of liver failure [54]. The system uses a hollow fibre bioreactor loaded with porcine hepatocytes.

Development of matrix-based hepatocyte transplantation

Over the last years, the injection of liver cell suspensions into anatomic structures such as the spleen [55], the kidney capsule [56] or the peritoneal cavity [57] has been performed in different animal models. Especially, the intraportal hepatocyte injection has been reported to be successful in animal models of metabolic deficiencies [58]. Recently, intraportal hepatocyte injection has been successfully applied on patients with the Crigler-Najjar syndrome type 1 [59]. However, portal hypertension, portal vein thrombosis and pulmonary embolism remain problematic when larger cell numbers are transplanted [60, 61].

Function and differentiation of liver cells are influenced by the three-dimensional organ architecture [62]. This concept led to combine cell transplantation with the application of three-dimensional, highly porous polymeric matrices as a concept of tissue engineering [63] (Fig. 2). It has several advantages when compared to the injection of cell suspensions into solid organs (Table 2). The matrices provide sufficient volume for the transplantation of cell numbers up to whole organ equivalents [64]. Transplantation efficiency could be improved by optimizing shape and composition of the matrices as well as by attaching growth factors and ECMs to the polymeric scaffold [23, 65]. Cell transplantation into polymeric matrices is, in contrast to cell injection into anatomic structures, a reversible procedure because the cell matrix constructs may be removed if so desired. In the future, this concept may even allow the construction of preservable, implantable liver support devices that are available without restrictions. The use of three-dimensional matrices as a carrier for the transplantation of genetically altered cells [66] is also conceivable [67]. Heterotopic hepatocyte transplantation in matrices has been demonstrated in long-term studies [68] (Fig. 3). Nevertheless, initial engraftment rates are suboptimal. In theory, the metabolic situation in patients with hepatic failure or other liver diseases may provide a hepatotrophic stimulus for hepatocytes in heterotopic locations *per se*.

Table 2 Achievements of heterotopic hepatocyte transplantation using 3D matrices

Goal	Comment	Reference
Whole liver mass transplantation		[64]
Coating of matrices with ECM molecules or attachment of growth factors	Increased cell engraftment and function	[23, 65]
Transplantation of genetically altered cells	Correction of metabolic defects	[66, 67]
Co-transplantation of different cell types	Increased hepatocyte survival and proliferation	[73, 74]
Long-term data after hepatocyte transplantation		[68]
Correction of vitamin C deficiency	ODS rat	[64]

ODS, osteogenic disorder Shionogi

However, such hepatotrophic effects could not be observed in animal models of metabolic enzyme deficiencies [69], which are considered the most important future indication for intracorporeal liver support devices. Because optimal transplantation efficiency is a prerequisite for any future clinical application, the improvement of engraftment and the continuous long-term stimulation of hepatocytes in the polymeric matrices are of great interest. Portocaval shunt operation in the recipient is a standard procedure for experimental long-term stimulation of hepatocytes in heterotopic sites [70], but the need for vascular surgery combined with the procedure-specific side effects [71] may reduce its applicability in humans. Selective segmental liver transplantation experiments by Starzl *et al*. [72] revealed that the majority of the hepatotrophic factors in the portal venous blood originate from the pancreatic circulation. Therefore, pancreatic islet co-transplantation seems to be an alternative for the stimulation of hepatocytes in polymeric matrices [73]. Co-transplantation of islets of Langerhans with hepatocytes and portocaval shunt supported engraftment of hepatocytes in polymeric matrices equally well. Islet cell-cotransplantation (ICT) did not interfere with the recipient's glucose metabolism and did not induce hyperproliferative premalignant foci within the transplanted hepatocytes. The technique is therefore an attractive approach towards hepatotrophic stimulation of BAL equivalents [74]. One of the main problems in hepatocyte transplantation – the vascularization and oxygenation of hepatocytes – may be solved by tissue engineering techniques. Recent improvements in the generation of prevascularized bone, fatty tissue and muscle structures are promising and may be transferable to the situation in hepatic tissue engineering [75–79]. In addition, recent data showed the possibility of creating a nanostructure in a capillary pattern by micro-electromechanical system (MEMS) [80]. In such a system a poly-glycol acid film was seeded with human umbilical vascular endothelial cells [81]. The combination of nanoscaffolds with successful prevascularization techniques is a promising tissue-engineered approach to provide sufficient vascularization of artificial tissue constructs for transplantation.

Outlook: future perspective for the development of successful tissue engineering approaches for transplantation

Because there is no sufficient supply of donor organs, and immunosuppression is associated with high morbidity and high costs, there is an urgent need for other therapeutic options. In some cases BAL devices and hepatocyte transplantation have been used with great success, but still have to be improved. Tissue engineering is one of the key techniques to hepatocyte transplantation and to BAL. Better understanding of cell culture techniques is necessary to achieve the goal of building effective liver support devices. First steps toward preformed and functional hepatic tissue with an organ-like microstructure have been made. Several cell types have been investigated for hepatocyte culture: stem cells, oval progenitor cells, mature hepatocytes and

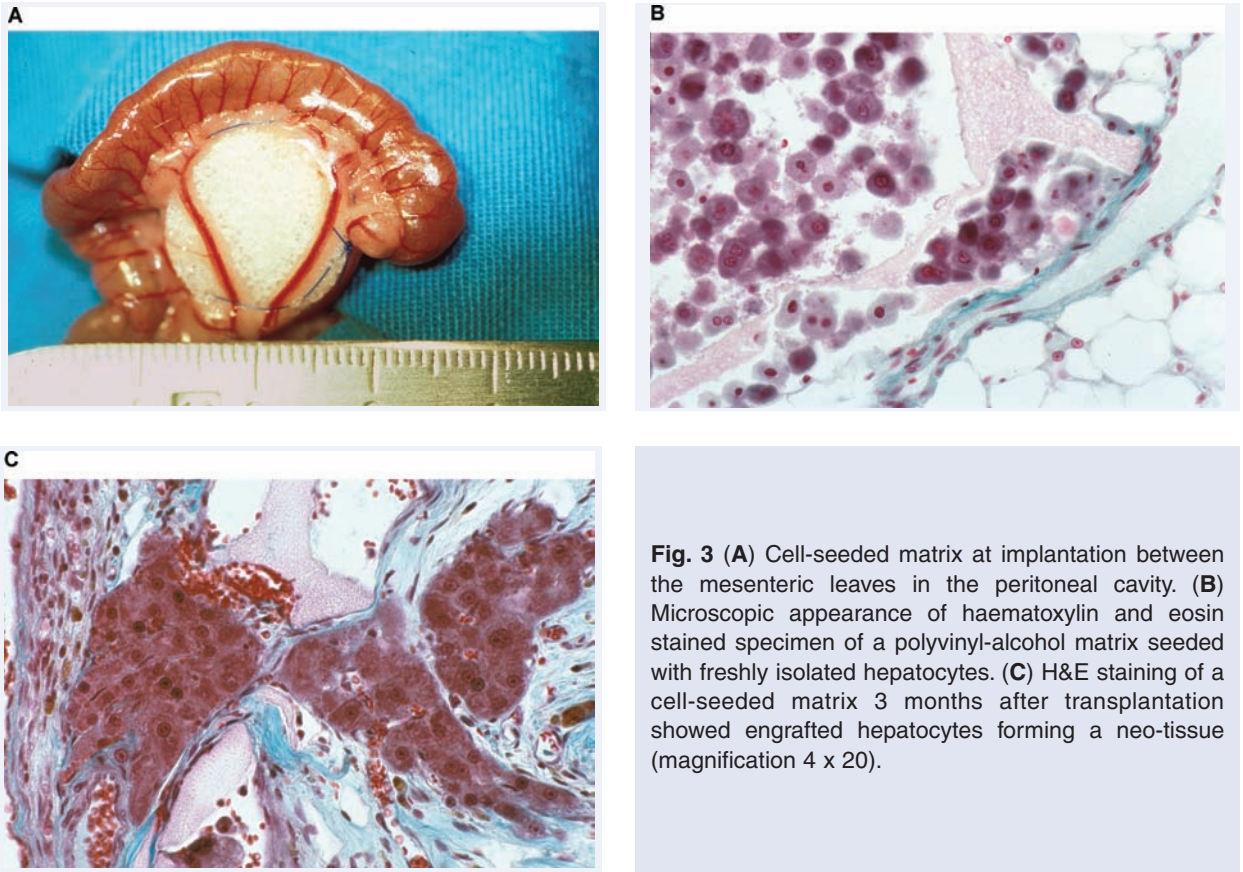

Fig. 3 (**A**) Cell-seeded matrix at implantation between the mesenteric leaves in the peritoneal cavity. (**B**) Microscopic appearance of haematoxylin and eosin stained specimen of a polyvinyl-alcohol matrix seeded with freshly isolated hepatocytes. (**C**) H&E staining of a cell-seeded matrix 3 months after transplantation showed engrafted hepatocytes forming a neo-tissue (magnification 4 x 20).

cells derived from *neo*plastic tissues of the liver. Still many problems have to be solved, but there is hope that cell-based therapies will be the standard therapy for metabolic diseases of the liver one day and that BAL may be as effective for FHF as dialysis is for kidney failure.

Acknowledgements

The authors would like to thank the medical students Christina Höper, Jana Ahrend and Daniel Schultze for their excellent laboratory work, and Beate Roth for technical assistance.

References

1. **Schiff L, Schiff E.** Diseases of the liver. 1st ed. Philadelphia, PA: J.B. *Lippincott;* 1993.
2. **Harper AM, Rosendale JD.** The UNOS OPTN waiting list and donor registry: 1988-1996. *Clin Transpl.* 1996; 10: 69–90.
3. **Fishman JA, Rubin RH.** Infection in organ-transplant recipients. *N Engl J Med.* 1998; 338: 1741–51.
4. UNOS-Database, http://www.unos.org/data, March 2007.
5. **Gerlach JC, Encke J, Hole O, Müller C, Ryan CJ, Neuhaus P.** Bioreactor for a larger scale hepatocyte *in vitro* perfusion. *Transplantation.* 1994; 58: 984–8.
6. **Raper SE.** Hepatocyte transplantation and gene therapy. *Clin Transplant.* 1995; 9: 249–54.
7. **Asonuma K, Gilbert JC, Stein JE, Takeda T, Vacanti JP.** Quantitation of transplanted hepatic mass necessary to cure the Gunn rat model of hyperbilirubinemia. *J Pediatr Surg.* 1992; 27: 298–301.
8. **Anderson NG.** The mass isolation of whole cells from rat liver. *Science.* 1953; 117: 627–8.
9. **Howard RB, Christensen AK, Gibbs FA, Pesch LA.** The enzymatic preparation of isolated intact parenchymal cells from rat liver. *J Cell Biol.* 1967; 35: 675–84.
10. **Berry MN, Friend DS.** High-yield preparation of isolated rat liver parenchymal cells: a biochemical and fine structural study. *J Cell Biol.* 1969; 43; 506–20.

11. **Seglen PO, Jervell KF.** A simple perfusion technique applied to glucocorticoid regulation of tryptophan oxygenase turnover and bile production in the isolated rat liver. *Hoppe Seylers Z Physiol Chem.* 1969; 350: 308–16.
12. **Liu XL, Li LJ, Chen Z.** Isolation and primary culture of rat hepatocytes. *Hepatobiliary Pancreat Dis Int.* 2002; 1: 77–9.
13. **Koenig S, Krause P, Drabent B, Schaeffner I, Christ B, Schwartz P, Unthan-Fechner K, Probst I.** The expression of mesenchymal, neural and haematopoietic stem cell markers in adult hepatocytes proliferating *in vitro*. *J Hepatol.* 2006; 44: 1115–24.
14. **Meredith MJ.** Rat hepatocytes prepared without collagenase: prolonged retention of differentiated characteristics in culture. *Cell Biol Toxicol.* 1988; 4: 405–25.
15. **Wang SR, Renaud G, Infante J, Catala D, Infante R.** Isolation of rat hepatocytes with EDTA and their metabolic functions in primary culture. *in vitro Cell Dev Biol.* 1985; 21: 526–30.
16. **Fiegel HC, Lange C, Kneser U, Lambrecht W, Zander AR, Rogiers X, Kluth D.** Fetal and adult liver stem cells for liver regeneration and tissue engineering. *J Cell Mol Med.* 2006; 10: 577–87.
17. **Lange C, Bassler P, Lioznov MV, Bruns H, Kluth D, Zander AR, Fiegel HC.** Liver-specific gene expression in mesenchymal stem cells is induced by liver cells. *World J Gastroenterol.* 2005; 11: 4497–504.
18. **Lange C, Bruns H, Kluth D, Zander AR, Fiegel HC.** Hepatocytic differentiation of mesenchymal stem cells in cocultures with fetal liver cells. *World J Gastroenterol.* 2006; 12: 2394–7.
19. **Ong SY, Dai H, Leong KW.** Inducing hepatic differentiation of human mesenchymal stem cells in pellet culture. *Biomaterials.* 2006; 27: 4087–97.
20. **Sato Y, Araki H, Kato J, Nakamura K, Kawano Y, Kobune M, Sato T, Miyanishi K, Takayama T, Takahashi M, Takimoto R, Iyama S, Matsunaga T, Ohtani S, Matsuura A, Hamada H, Niitsu Y.** Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. *Blood.* 2005; 106: 756–63.
21. **Moviglia GA, Varela G, Gaeta CA, Brizuela JA, Bastos F, Saslavsky J.** Autoreactive t cells induce *in vitro* BM mesenchymal stem cell transdifferentiation to neural stem cells. *Cytotherapy.* 2006; 8: 196–201.
22. **Kaufmann PM, Sano K, Uyama S, Takeda T, Vacanti JP.** Heterotopic hepatocyte transplantation: assessing the impact of hepatotrophic stimulation. *Transplant Proc.* 1994; 26: 2240–1.
23. **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.
24. **Berthiaume F, Moghe PV, Toner M, Yarmush ML.** Effect of extracellular matrix topology on cell structure, function, and physiological responsiveness: hepatocytes cultured in a sandwich configuration. *FASEB J.* 1996; 10: 1471–84.
25. **Block GD, Locker J, Bowen WC, Petersen BE, Katyal S, Strom SC, Riley T, Howard TA, Michalopoulos GK.** Population expansion, clonal growth, and specific differentiation patterns in primary cultures of hepatocytes induced by HGF/SF, EGF and TGF alpha in a chemically defined (HGM) medium. *J Cell Biol.* 1996; 132: 1133–49.
26. **Reid LM.** Stem cell biology, hormone/matrix synergies and liver differentiation. *Curr Opin Cell Biol.* 1990; 2: 121–30.
27. **Guguen-Guillouzo C, Clément B, Baffet G, Beaumont C, Morel-Chany E, Glaise D, Guillouzo A.** Maintenance and reversibility of active albumin secretion by adult rat hepatocytes co-cultured with another liver epithelial cell type. *Exp Cell Res.* 1983; 143: 47–54.
28. **Shimaoka S, Nakamura T, Ichihara A.** Stimulation of growth of primary cultured adult rat hepatocytes without growth factors by coculture with nonparenchymal liver cells. *Exp Cell Res.* 1987; 172: 228–42.
29. **Bhatia SN, Balis UJ, Yarmush ML, Toner M.** Effect of cell-cell interactions in preservation of cellular phenotype: cocultivation of hepatocytes and nonparenchymal cells. *FASEB J.* 1999; 13: 1883–900.
30. **Mizuguchi T, Hui T, Palm K, Sugiyama N, Mitaka T, Demetriou AA, Rozga J.** Enhanced proliferation and differentiation of rat hepatocytes cultured with bone marrow stromal cells. *J Cell Physiol.* 2001; 189: 106–19.
31. **Rozga J, Williams F, Ro MS, Neuzil DF, Giorgio TD, Backfisch G, Moscioni AD, Hakim R, Demetriou AA.** Development of a bioartificial liver: properties and function of a hollow-fiber module inoculated with liver cells. *Hepatology.* 1993; 17: 258–65.
32. **Kaufmann PM, Heimrath S, Kim BS, Mooney DJ.** Highly porous polymer matrices as a three-dimensional culture system for hepatocytes. *Cell Transplant.* 1997; 6: 463–8.
33. **Török E, Pollok JM, Ma PX, Vogel C, Dandri M, Petersen J, Burda MR, Kaufmann PM, Kluth D, Rogiers X.** Hepatic tissue engineering on 3-dimensional biodegradable polymers within a pulsatile flow bioreactor. *Dig Surg.* 2001; 18: 196–203.
34. **Pearse MJ, Witort E, Mottram P, Han W, Murray-Segal L, Romanella M, Salvaris E, Shinkel TA,**

Goodman DJ, d'Apice AJ. Anti-gal antibody-mediated allograft rejection in alpha1,3-galactosyltransferase gene knockout mice: a model of delayed xenograft rejection. *Transplantation.* 1998; 66: 748–54.

35. **Obermayer N, Busse B, Grünwald A, Mönch E, Müller C, Neuhaus P, Gerlach JC.** Biochemical characterization of bioreactors for hybrid liver support: serum-free liver cell coculture of nonparenchymal and parenchymal cells. *Transplant Proc.* 2001; 33: 1930–1.
36. **Bartolo LD, Bader A.** Flat membrane bioreactor for the replacement of liver functions. *Ernst Schering Res Found Workshop.* 2002; 35: 89–104.
37. **Fiegel HC, Bruns H, Höper C, Lioznov MV, Kluth D.** Cell growth and differentiation of different hepatic cells isolated from fetal rat liver *in vitro*. *Tissue Eng.* 2006; 12: 123–30.
38. **Yanagi K, Ookawa K, Mizuno S, Ohshima N.** Performance of a new hybrid artificial liver support system using hepatocytes entrapped within a hydrogel. *ASAIO Trans.* 1989; 35: 570–2.
39. **Doré E, Legallais C.** A new concept of bioartificial liver based on a fluidized bed bioreactor. *Ther Apher.* 1999; 3: 264–7.
40. **O'Grady JG, Gimson AE, O'Brien CJ, Pucknell A, Hughes RD, Williams R.** Controlled trials of charcoal hemoperfusion and prognostic factors in fulminant hepatic failure. *Gastroenterology.* 1988; 94: 1186–92.
41. **Stange J, Mitzner S, Ramlow W, Gliesche T, Hickstein H, Schmidt R.** A new procedure for the removal of protein bound drugs and toxins. *ASAIO J.* 1993; 39: M621–5.
42. **Sorrentino F.** Prime ricerche per la realizzatione di un fegato artificiale. *Chir Patol Sper.* 1956; 4: 1401.
43. **Wolf CF, Munkelt BE.** Bilirubin conjugation by an artificial liver composed of cultured cells and synthetic capillaries. *Trans Am Soc Artif Intern Organs.* 1975; 21: 16–27.
44. **Nyberg SL, Remmel RP, Mann HJ, Peshwa MV, Hu WS, Cerra WF.** Primary hepatocytes outperform Hep-G2 cells as the source of biotransformation functions in a bioartificial liver. *Ann Surg.* 1994; 220: 59–67.
45. **Sussman NL, Gislason GT, Conlin CA, Kelly JH.** The hepatic extracorporeal liver assist device: initial clinical experience. *Artif Organs.* 1994; 18: 390–6.
46. **Millis MJ, Cronin DC, Johnson R, Conjeevaram H, Conlin C, Trevino S, Maguire P.** Initial experience with the modified extracorporeal liver-assist device for patients with fulminant hepatic failure: system modifications and clinical impact. *Transplantation.* 2002; 74: 1735–46.
47. **Demetriou AA, Brown RS, Busuttil RW, Fair J, McGuire BM, Rosenthal P, Esch JSA, Lerut J, Nyberg SL, Salizzoni M, Fagan EA, de Hemptinne B, Broelsch CE, Muraca M, Salmeron JM, Rabkin JM, Metselaar HJ, Pratt D, Mata MDL, McChesney LP, Everson GT, Lavin PT, Stevens AC, Pitkin Z, Solomon BA.** Prospective, randomized, *multi*center, controlled trial of a bioartificial liver in treating acute liver failure. *Ann Surg.* 2004; 239: 660–7.
48. **Stadlbauer V, Jalan R.** Acute liver failure: liver support therapies. *Curr Opin Crit Care.* 2007; 13: 215–21.
49. **Sauer IM, Zeilinger K, Obermayer N, Pless G, Grünwald A, Pascher A, Mieder T, Roth S, Goetz M, Kardassis D, Mas A, Neuhaus P, Gerlach JC.** Primary human liver cells as source for modular extracorporeal liver support – a preliminary report. *Int J Artif Organs.* 2002; 25: 1001–5.
50. **Sauer IM, Kardassis D, Zeillinger K, Pascher A, Gruenwald A, Pless G, Irgang M, Kraemer M, Puhl G, Frank J, Müller AR, Steinmüller T, Denner J, Neuhaus P, Gerlach JC.** Clinical extracorporeal hybrid liver support – phase I study with primary porcine liver cells. *Xenotransplantation.* 2003; 10: 460–9.
51. **Mundt A, Puhl G, Müller A, Sauer I, Müller C, Richard R, Fotopoulou C, Doll R, Gäbelein G, Höhn W, Hofbauer R, Neuhaus P, Gerlach J.** A method to assess biochemical activity of liver cells during clinical application of extracorporeal hybrid liver support. *Int J Artif Organs.* 2002; 25: 542–8.
52. **Flendrig LM, la Soe JW, Jörning GG, Steenbeek A, Karlsen OT, Bovée WT, Ladiges NC, te Velde AA, Chamuleau RA.** *In vitro* evaluation of a novel bioreactor based on an integral oxygenator and a spirally wound nonwoven polyester matrix for hepatocyte culture as small aggregates. *J Hepatol.* 1997; 26: 1379–92.
53. **Van de Kerkhove MP, Florio ED, Scuderi V, Mancini A, Belli A, Bracco A, Dauri M, Tisone G, Nicuolo GD, Amoroso P, Spadari A, Lombardi G, Hoekstra R, Calise F, Chamuleau RA.** Phase I clinical trial with the AMC-bioartificial liver. *Int J Artif Organs.* 2002; 25: 950–9.
54. **Patzer JF, Mazariegos GV, Lopez R, for the RAP Investigators.** Preclinical evaluation of the Excorp Medical, Inc, bioartificial liver support system. *J Am Coll Surg.* 2002; 195: 299–310.
55. **Mito M, Ebata H, Kusano M, Onishi T, Saito T, Sakamoto S.** Morphology and function of isolated hepatocytes transplanted into rat spleen. *Transplantation.* 1979; 28: 499–505.
56. **Xiangdong W, Ar'Rajab A, Ahrén B, Andersson R, Bengmark S.** The effect of pancreatic islets on

transplanted hepatocytes in the treatment of acute liver failure in rats. *Res Exp Med.* 1991; 191: 429–35.

57. **Demetriou AA, Whiting JF, Feldman D, Levenson SM, Chowdhury NR, Moscioni AD, Kram M, Chowdhury RJ.** Replacement of liver function in rats by transplantation of microcarrier-attached hepatocytes. *Science.* 1986; 233: 1190–2.
58. **Holzman MD, Rozga J, Neuzil DF, Griffin D, Moscioni AD, Demetriou AA.** Selective intraportal hepatocyte transplantation in analbuminemic and Gunn rats. *Transplantation.* 1993; 55: 1213–9.
59. **Fox IJ, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI, Dorko K, Sauter BV, Strom SC.** Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. *N Engl J Med.* 1998; 338: 1422–6.
60. **Nieto JA, Escandón J, Betancor C, Ramos J, Cantón T, Cuervas-Mons V.** Evidence that temporary complete occlusion of splenic vessels prevents massive embolization and sudden death associated with intrasplenic hepatocellular transplantation. *Transplantation.* 1989; 47: 449–50.
61. **Benedetti E, Kirby JP, Asolati M, Blanchard J, Ward MG, Williams R, Hewett TA, Fontaine M, Pollak R.** Intrasplenic hepatocyte allotransplantation in dalmatian dogs with and without cyclosporine immunosuppression. *Transplantation.* 1997; 63: 1206–9.
62. **Inber DE.** Extracellular matrix and the development of tissue architecture: a mechanochemical perspective. In: Zern MA, Reid LM, editors. Extracellular matrix. *New York: Marcel Dekker, Inc.;* 1993. pp. 403–28.
63. **Langer R, Vacanti JP.** Tissue engineering. *Science.* 1993; 260: 920–6.
64. **Uyama S, Kaufmann PM, Kneser U, Fiegel HC, Pollok JM, Kluth D, Vacanti JP, Rogiers X.** Hepatocyte transplantation using biodegradable matrices in ascorbic acid-deficient rats: comparison with heterotopically transplanted liver grafts. *Transplantation.* 2001; 71: 1226–31.
65. **Hkrach JS, Ou J, Lothan N, Langer R.** Poly(l-lactic acid-co-aspartic acid): interactive polymers for tissue engineering. Polymers in medicine and pharmacy. Symposium Proceedings 394, A.G. In: Mikos KW, Leong MJ, Yaszemski JA, Tamada ML, Radomsky KW, editors. Pittsburgh, PA: *Materials Research Society;* 1995: pp. 77–82.
66. **Bleiziffer O, Eriksson E, Yao F, Horch RE, Kneser U.** Gene transfer strategies in tissue engineering. *J Cell Mol Med.* 2007; 11: 206–23.
67. **Gilbert JC, Takada T, Stein JE, Langer R, Vacanti JP.** Cell transplantation of genetically altered cells on biodegradable polymer scaffolds in syngeneic rats. *Transplantation.* 1993; 56: 423–7.
68. **Kaufmann PM, Kneser U, Fiegel HC, Kluth D, Herbst H, Rogiers X.** Long-term hepatocyte transplantation using three-dimensional matrices. *Transplant Proc.* 1999; 31: 1928–9.
69. **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: 273–81.
70. **Mooney DJ, Sano K, Kaufmann PM, Majahod K, Schloo B, Vacanti JP, Langer R.** Long-term engraftment of hepatocytes transplanted on biodegradable polymer sponges. *J Biomed Mater Res.* 1997; 37: 413–20.
71. **Harley HA, Morgan T, Redeker AG, Reynolds TB, Villamil F, Weiner JF, Yellin A.** Results of a randomized trial of end-to-side *portacaval* shunt and distal splenorenal shunt in alcoholic liver disease and variceal bleeding. *Gastroenterology.* 1986; 91: 802–9.
72. **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: 179–99.
73. **Kaufmann PM, Kneser U, Fiegel HC, Pollok JM, Kluth D, Izbicki JR, Herbst H, Rogiers X.** Is there an optimal concentration of cotransplanted islets of Langerhans for stimulation of hepatocytes in three dimensional matrices? *Transplantation.* 1999; 68: 272–9.
74. **Kneser U, Kaufmann PM, Fiegel HC, Pollok JM, Rogiers X, Kluth D, Herbst H.** Interaction of hepatocytes and pancreatic islets cotransplanted in polymeric matrices. *Virchows Arch.* 1999; 435: 125–32.
75. **Kneser U, Polykandriotis E, Ohnolz J, Heidner K, Grabinger L, Euler S, Amann KU, Hess A, Brune K, Greil P, Stürzl M, Horch RE.** Engineering of vascularized transplantable bone tissues: induction of axial vascularization in an osteoconductive matrix using an arteriovenous loop. *Tissue Eng.* 2006; 12: 1721–31.
76. **Polykandriotis E, Arkudas A, Horch RE, Stürzl M, Kneser U.** Autonomously vascularized cellular constructs in tissue engineering: opening a new perspective for biomedical science. *J Cell Mol Med.* 2007; 11: 6–20.
77. **Bach AD, Arkudas A, Tjiawi J, Polykandriotis E, Kneser U, Horch RE, Beier JP.** A new approach to tissue engineering of vascularized skeletal muscle. *J Cell Mol Med.* 2006; 10: 716–26.
78. **Kneser U, Stangenberg L, Ohnolz J, Buettner O, Stern-Straeter J, Möbest D, Horch RE, Stark GB, Schaefer DJ.** Evaluation of processed bovine cancellous bone matrix seeded with syngenic osteoblasts in a critical size calvarial defect rat model. *J Cell Mol Med.* 2006; 10: 695–707.

79. **Horch RE.** Future perspectives in tissue engineering. *J Cell Mol Med.* 2006; 10: 7–19.
80. **Fidkowski C, Kaazempur-Mofrad MR, Borenstein J, Vacanti JR, Langer R, Wang Y.** Endothelialized microvasculature based on a biodegradable elastomer. *Tissue Eng.* 2005; 11: 302–9.
81. **Shin M, Matsuda K, Ishii O, Terai H, Kaazempur-Mofrad MR, Borenstein J, Vacanti JP.** Endothelialized networks with a vascular geometry in microfabricated poly (dimethyl siloxane). *Biomed Microdevices.* 2004; 6: 269–78.
82. **Mazariegos GV, Kramer DJ, Lopez RC, Obaid Shakil A, Rosenbloom AJ, DeVera M, Giraldo M, Grogan TA, Zhu Y, Fulmer ML, Amiot BP, Patzer JF.** Safety observations in phase I clinical evaluation of the Excorp medical bioartificial liver support system after the first four patients. *ASAIO J.* 2001; 47: 471–5.