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Prospects of the use of hepatic cells for extracorporeal liver support

Jörg C. Gerlach

Dept. of Surgery and Bioengineering, McGowan Institute for Regenerative Medicine, University of Pittsburgh, PA, USA; and Div. of Experimental Surgery, Dept. of Surgery, Charite - Campus Virchow, Humboldt University Berlin, Germany.

Abstract

Hybrid extracorporeal liver support is an option to assist liver transplantation therapy. An overview on liver cell bioreactors is given and our own development is described. Furthermore, the prospects of the utilization of human liver cells from discarded transplantation organs due to steatosis, cirrhosis or traumatic injury, and liver progenitor cells are discussed. Our Modular Extracorporeal Liver Support (MELS) concept proposes an integrative aproach for the treatment of hepatic failure with appropriate extracorporeal therapy units, tailored to suit the actual clinical needs of each patient. The *CellModule* is a specific bioreactor (charged actually with primary human liver cells, harvested from human donor livers found to be unsuitable for transplantation). The *DetoxModule* enables albumin-dialysis for the removal of albumin-bound toxins, reducing the biochemical burden of the liver cells, and replacing the bile excretion of hepatocytes in the bioreactor. A *Dialysis Module* for continuous veno-venous hemofiltration can be added to the system if required in hepato-renal syndrome. (Acta gastroenterol. belg., **2005**, 68, **358-368**).

Key words : liver support, bioreactors, primary human liver cells, liver progenitor cells.

Prospects of the use of hepatic cells for extracorporeal liver support

Patients with liver disease, fulminant and chronic, can progressively worsen until they require orthotopic liver transplantation (OLT), which is the only recognized effective treatment (1). The American Liver Foundation estimates that one in ten people have some form of liver disease and 26,000 people die each year from liver disease (2). In 2002, 16,929 people were listed for liver transplantation in the US, only 4,778 cadaveric donor liver transplants were made, and 1,756 patients died while awaiting transplant (3). A medical need for a treatment modality that can “bridge” patients to transplant or slow or reverse the progression of acute liver disease is clearly demanded.

Acute liver failure (ALF) has a poor prognosis with mortality rates between 50% and 90% under conservative management (4). Over the last 50 years, advances have been made in the understanding of the pathophysiology encompassing the clinical features of ALF : encephalopathy, cerebral edema, hemorrhage, electrolyte and metabolic disturbances, renal failure, cardiovascular instability, and increased risk of infection. While cerebral edema is the most common cause of death, multi-organ failure and sepsis are also associated with significant mortality. Depending on etiology, the survival rate in acute liver failure under conservative treatment ranges from 79% (amanita intoxication) to 10% (cryptogeneic genesis). Introduction of liver transplantation (LTx) as therapeutic option reduced mortality to 20-40% (5).

With the continued, growing disparity between the numbers of organ donations and patients waiting for liver transplantation, efforts have been made to design extracorporeal methods to support or replace the failing liver. The frequent lack of donor livers for urgent transplantation in ALF highlights the need for a liver support therapy until an organ becomes available. Moreover, patients with the capacity for liver recovery could be bridged to regeneration and would not require transplantation at all.

Progress in hepatocyte tissue engineering and *in vitro* maintenance of differentiated function of primary liver cells has led to initial clinical pilot studies of extracorporeal support of patients in acute liver failure using bioreactors incorporating hepatocyte cultures (6,7,8,9, 10,11). While encouraging, the nonsignificant results from the only Phase 3 efficacy trial conducted to date (12) indicates that much further progress in understanding and controlling tissue engineering of complex hepatocyte bioreactors is required. One of the challenges is the creation of neo-vascularized tissue constructs at high cell density that avoid central necrosis and exhibit recapitulation of the sinusoidal microvasculature of the liver.

Liver Support with Bioreactors

Table 1 summarizes literature, describing bioreactor constructions for extracorporeal liver support, tested in animal experiments ; table 2 summarized constructions which were used clinically.

Previously, we focused on a spontaneous re-assembly of primary cells inoculated into a bioreactor and their establishment of a scaffold or biomatrix. We have shown that a homogeneous mix of adult liver cells from organ collagenase digestion containing parenchymal hepatocytes, non-parenchymal cells such as sinusoidal endothelial cells, stellate cells, and liver progenitor cells

Correspondence : Jörg C. Gerlach M.D., Ph.D., Professor of Surgery – School of Medicine, Dept. of Surgery, Professor of Bioengineering – School of Engineering, University of Pittsburgh ; McGowan Institute for Regenerative Medicine, Bridgeside Point Bldg., 100 Technology Drive, Suite 225, Pittsburgh, PA 15219-3130 USA.
E-mail : jgerlach@piitt.edu.

Table 1. — **Bioreactor Constructions Tested in Animal Models (Hout & Gerlach)**

Technology	Cell Type	Citation
Hollow fiber-based bioartificial liver with integral oxygenation (Gerlach CellModule)	Porcine liver cells	Gerlach *et al.*, 1993 (13), 1994 (14), 2001 (15); Janke *et al.*, 1997 (16)
Spirally wound flat sheet and hollow fiber-based bioartificial liver with integral oxygenation	Porcine hepatocytes	Flendrig *et al.*, 1999 (17)
Flat plate bioartificial liver with integral oxygenation	Porcine hepatocytes	Shito *et al.*, 2003 (18)
Hollow fiber-based renal tubule assist device	Human renal tubule cells	Humes *et al.*, 2002 (19)

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Fig. 1. — Clinical use of our 600 gm cell compartment bioreactor. (Gerlach & Sauer, Charité, Berlin, Germany).

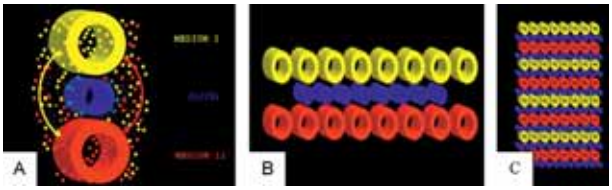

Fig. 2. — Schematic depiction of the four-compartment stem cell bioreactor. A) Smallest repeating cell culture unit within the bioreactor. B) Analytical scale stem cell bioreactor. C) Laboratory scale stem cell bioreactor. (McKeel & Gerlach).

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will restructure after injection into a bioreactor to form well-defined liver structures, such as neo-sinusoidal structures and neo-space of Dissé, reminiscent of the native liver. These studies were conducted in bioreactors with 800 grams of cells used for extracorporeal liver support (Fig. 1).

Bioreactors that are currently used for primary liver cell culture and clinical liver support are hollow fiber based and exhibit two functional compartments (13,14). Xu *et al*. pointed out, that four bioreactor compartments are necessary to enable integral oxygenation and distributed mass exchange with low gradients (15). We took this challenge and developed a bioreactor specific for clinical liver support that accommodated 400-800 g of primary cells (16) (Fig. 2).

The vasculature of organs can be regarded as complex structures which supply plasma to the cells surrounding the network of capillaries. Oxygen supply is enhanced by the perfusion of hemoglobin-containing erythrocytes. Mass exchange is enhanced by pulsation in blood flow and alterations in the autonomous capillary resistance. Using various independently perfused interwoven networks of hollow fibers, our approach was to mimic the native structure of an organ using an artificial capillary bed. We addressed the lack of erythrocytes by flowing oxygen through one compartment. Mass exchange was addressed by perfusing two independent capillary systems with plasma and media. Small artificial hollow fiber capillary subunits, in which interwoven membranes represent the organs secondary level of vascular supply, are simultaneously perfused.

The design focused on transforming the previously published bioreactor constructions into a technology with up to five compartments. A construction principle was developed to form independently perfused subunits from woven synthetic capillaries, each with a different function, and arrange them in parallel (17). To scale-up the bioreactor, additional subunits are added resulting in a capillary system (18,19), similar to the natural organ microvasculature. The extracapillary space forms a compartment were the inoculated liver cells and hepatocyte nursing cells (20,21,22,23) reside (24). Cell migration processes reform tissue-like structures between the capillaries. Under conditions of capillary perfusion, parenchymal and non-parenchymal tissue formation (25) is possible by spontaneous organization to cellular aggregates and their attachment to the surface of the capillaries (26,27).

Human liver cells in bioreactors for liver support

A key question in the development of cell-based liver support concerns the cell source. Primary cells have the advantage of differentiated function. Initial clinical studies employing primary porcine liver cells for extracorporeal liver support were performed by others and by our group (28,29). However, the use of primary porcine cells, which are used in most of the current technologies, is controversial for various reasons, including the possible transfer of porcine endothelial retroviruses and possible immunologic reactions by the xenogeneic proteins produced (30).

Table 2. — **Bioreactor Constructions Tested *In Vitro* (Hout & Gerlach)**

Technology	Cell Type	Citation
Early perfusion chambers	Chick heart fibroblasts, human malignant epithelial cells, Chinese hamster cells, hybridomas	Christiansen *et al.*, 1953 ([1]) ; Rose, 1954 ([2]) ; Freed, 1963 ([3]) ; Katinger, 1985 ([4])
Commercially available perfusion chambers	Bone marrow-derived osteoblasts	Minucells ([5])
Commercially available systems for non-adherent cells	Hybridomas	VectraCell gas-permeable bags ([6]), Rotary Cell Culture System ([7]), Wave Bioreactor ([8]), CELLine ([9]), miniPERM Bioreactor ([10]), CellMax ([11]), Tecnomouse ([12])
Commercially available system for bone marrow expansion	Hematopoetic stem cells	AastromReplicell ([13])
Hollow fiber-based bioreactors	Mouse fibroblasts, human choriocarcinoma cells, Reuber hepatoma cells, human hepatocytes	Knazek *et al.*, 1972 ([14]) ; Wolf *et al.*, 1975 ([15]) ; Hager *et al.*, 1978 ([16]), 1983 ([17])
Hollow fiber-based bioreactor with integral oxygenation	Human leukemic cell lines	Gloeckner *et al.*, 2001 ([18])
Coaxial hollow fiber-based bioreactor with integral oxygenation	Rat hepatocytes	Macdonald *et al.*, 2001 ([19])
Hollow fiber-based bioartificial liver with integral oxygenation (Gerlach CellModule)	Porcine and human liver cells	Gerlach *et al.*, 1994 ([20]), 1996 ([21]), 2003 ([22,23]) ; Sauer *et al.*, 2002 ([24])
Flat sheet and hollow fiber-based bioartificial liver with integral oxygenation	Porcine hepatocytes	Flendrig *et al.*, 1997 ([25])
Hollow fiber-based renal tubule assist device (Humes RAD)	Porcine renal tubule cells	Humes *et al.*, 1999 ([26])
Flat membrane bioreactor with integral oxygenation	Porcine hepatocytes	De Bartolo *et al.*, 2000 ([27])
Flat plate bioartificial liver with integral oxygenation	Porcine hepatocytes	Shito *et al.*, 2001 ([28])
Micropatterned borosilicate wafers	Rat hepatocytes and 3T3 fibroblasts	Bhatia *et al.*, 1997 ([29])
Biodegradable polymer bioreactor constructed via 3D printing	Rat liver cells	Kim *et al.*, 1998 ([30])
Microfabricated bioreactor constructed via ion etching of silicon wafers	Rat hepatocytes	Powers *et al.*, 2002 ([31,32])
Titanium mesh bioreactor	Rat bone marrow stromal osteoblasts	Bancroft *et al.*, 2003 ([33])
Bioreactor containing hydrated polyester fibers and porcine autologous biomatrix	Porcine hepatocytes (10^{10})	Ambrosino *et al.*, 2002 ([34])
Bioreactor containing nonwoven polyurethane matrix with integral oxygenation	Rat or pig hepatocytes	Linti *et al.*, 2002 ([35])

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The question of cell source for extracorporeal liver support has been subject to controversial discussions. A multivariate analysis of variance of patients treated with extracorporeal liver perfusion between 1964 and 2001 revealed that only the use of baboon and human livers provides an independent positive prognostic marker for improved survival (31). This analysis supports the thesis that xenogenic hepatocytes are not an optimal substitute for the complex tasks of a human liver. It is necessary to consider that in the studies cited, human livers of impaired quality and therefore unsuitable for LTx are compared with porcine livers of ideal quality. In order to avoid the drawbacks involved in using porcine cells and human liver tumor cell lines, the use of primary human liver cells seems to present a promising cell source.

Primary human liver cells, obtained from explanted organs found to be unsuitable for transplantation, are an interesting cell source as they are ethically acceptable and are capable of performing human metabolism and regulation (32). According to Eurotransplant data, and the data of the European and American organ procurement organizations, approximately 20-25% of all explanted livers are unsuitable for transplantation and therefore discarded. This number corresponds to the number of patients with acute liver failure (ALF) requiring bridging to liver transplantation (LTx) (33). However, the initial viability of those cells is impaired by preceding organ preservation and the isolation procedure.

Our hypothesis is that, with appropriate logistics, after recovery from the preservation & isolation injury in a four-compartment bioreactor, cells from these organs could serve the demand for cell-based therapy, including extracorporeal liver support.

In one of our studies, cells were isolated from 54 human livers from discarded transplants. A cell mass of 400-600 g was obtained enabling the clinical application of a liver lobe equivalent to a hybrid organ. Freshly isolated cell preparations contained about 10% of non-parenchymal cells, as estimated microscopically using size-selective criteria. 18 cell isolation procedures (33.3%) failed because of unsatisfactory cell viability (< 40%) and/or unsuccessful separation during the washing procedure, due to a higher grade of initial organ impairment. In 36 cases (66.7%), the isolation was performed successfully. From these isolation procedures, 2.8 to 6.4 $\times$ 10^{10} hepatocytes were co-cultured in the bioreactors (n = 36) with the non-parechymal cells of the same liver. Trypan blue viability of the cells prior to charging the bioreactors was 55.0 $\pm$ 15.9%. After inoculation into bioreactors, metabolic activities of the cells *in vitro* were maintained over at least 3 weeks of culturing.

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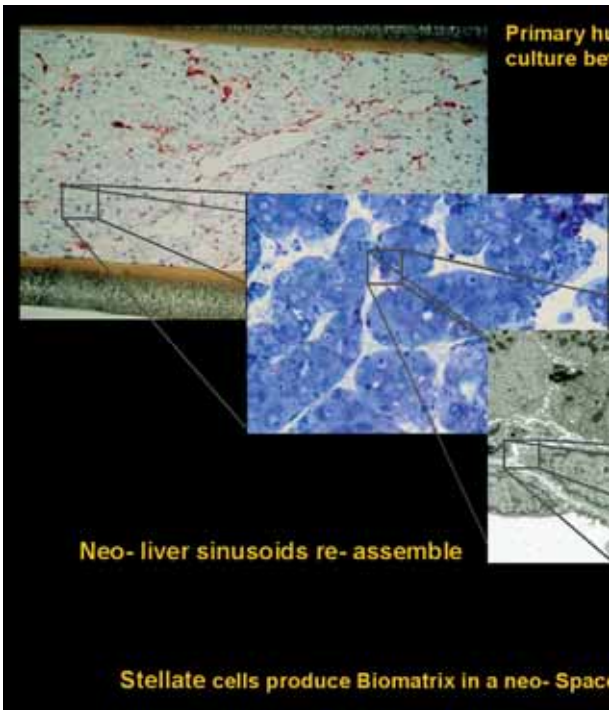

Fig. 3. — Microscopic "zoom" through the self-reassembled tissue structures formed by human primary liver cells in a four compartment hollow fiber based bioreactor.

Bioreactor culture of liver cells and clinical applications of our own development

Maintenance of non dividing primary liver cells and their differentiated functions was demonstrated over a period of two months and may continue longer (34,35,36). This bioreactor can accommodate a number of cells that would allow studies in extracorporeal liver support (37,38,39).

Animal studies showed encouraging results for the clinical use of the bioreactor (40,41,42,43,44,45).

Utilizing porcine cells, the device was used as a bridge to liver transplantation in a phase-I study with 8 patients in acute liver failure, coma stage III-IV (46). To date the patient & organ survival rate is 100% with an observation period of 3 years (47,48).

A pilot study in Berlin, Germany, is using the device with primary human liver cells harvested from organs explanted for transplantation but subsequently discarded due to steatosis, cirrhosis or mechanical injury (49). These results demonstrate the feasibility of employing primary human liver cells in clinical applications with specific bioreactor systems, and that the non-dividing cells recover from organ preservation and cell isolation injury (50,51).

This novel technology enabled spontaneous cell reorganization in the 600g sample as well as human tissue function over the period of several weeks (Fig. 3). By replacing the secondary capillary structure of the liver lobuli with artificial hollow fibers for nutrition, mass exchange and oxygenation (52), the cells spontaneously re-formed the primary capillary structure of liver sinusoids thus supporting a larger cell mass at a higher density (53). However, the limiting factor of liver support still remains the availability of primary human cells, as the present source is from discarded organs intended for transplantation. Thus, although an attractive technology with therapeutic potential is available, the availability of the cell source is crucial to enable further clinical studies. Stem cell research seeks to provide therapeutic solutions for this problem.

Liver progenitor cells and bioreactor cultures to study cell sources for extracorporeal liver support

Stem cells – unspecialized cells that perpetuate themselves through self-renewal and generate specialized cells through differentiation (54) – may be the ideal cell source for cell-based therapies developed to treat debilitating and life-threatening diseases (55).

Stem cells are characterized by their potential to differentiate into mature cell types that may have therapeutic benefit in the support, repair, and regeneration of tissues damaged by disease or trauma (56).

Cell-based therapies involve the transplantation of healthy cells to replace damaged cells (to treat diseases such as liver disease (57), heart disease (58), diabetes (59), Parkinson's disease (60), and muscular dystrophy (61)), or the use of bioartificial organs to support patients until organ recovery or whole organ transplantation (to treat diseases such as liver disease (62) and kidney disease (63)).

To realize the full therapeutic potential of stem cells, many investigators are seeking *in vitro* methods for maintaining the unspecialized state of stem cells, promoting the proliferation of stem cells, and directing the differentiation of stem cells to the needed specialized cells.

Objective of our work was to down scale the four-compartment 3D stem cell bioreactor technology platform to study embryonic, fetal, and adult stem cells in a tightly controlled environment (Fig. 2). We have cultured fetal liver stem cells in the bioreactors. During development, hepatic induction in mice begins as early as E9 and a liver bud is recognizable at this stage (64,65). In collaboration with the laboratory of Paul Monga, Dept. of Pathology at the University of Pittsburgh, we have utilized microscopically dissected embryonic livers from different stages of liver development including E10 through E14, and performed collagenase cell dissociation. A progenitor pool was maintained over at least three weeks, exhibiting a growing cell mass, differenciation towards liver tissue while the early progenitors were also increasing in number. Initial bioreactor cultures of human fetal liver cell preparations exhibited comparable results to those of mice fetal liver cells.

There are several applications for stem cell related research in the development of new products in medicine. Further advancement of stem cell-related research may evolve new therapies in transplantation medicine such as cell transplantation and extracorporeal bioreactor application for temporary support of a failing organ. In selected indications initial efforts are being made to replace the actual organ transplantation with adult cell transplantation. However, stem cell transplantation may be more suitable then transplantation of the fully differentiated adult derivatives, because of better retention of the ability to form junctions and communicate with host cells.

Challenges regarding stem cell research for liver support development

The current challenge that prevents stem cells from use in these applications is directing the full differentiation of their progeny *in vitro*. In order to further develop new therapies, adult and embryonic stem cell research focuses on maintenance, proliferation and differentiation, and tissue formation *in vitro*. Numerous groups already work on derivation and characterization of specific stem cell lineages, but the underlying mechanisms are only partly understood.

Several fundamental questions remain to be answered, e.g. :

a) Can we control proliferation/differentiation of selected stem cells *in vitro* ?
b) Can we maintain the genotype/phenotype stability of selected stem cells *in vitro* ?
c) Can specific microenvironmental conditions be used to control in vitro maintenance of selected stem cells ?
d) Can a phenotypic stabilization of selected stem cells be achieved by 3D high-density co-culture with integrated oxygenation and decentralized mass exchange ?
e) Can, after inoculation of selected isolated stem cells, tissue restructuring be achieved by the cells themselves ?
f) Can tissue formation by selected stem cells be induced and controlled in vitro ?
g) Can we establish in vitro a tissue-density of a larger number of selected stem cells without central necrosis ?
h) Can a reproducible proliferation/differentiation and utilization of selected stem cells be achieved in vitro ?
i) Can specific macroenvironmental conditions, such as 3D high-density co-culture with integrated oxygenation and decentralized mass exchange, be used to control *in vitro* maintenance of human embryonic stem cells in a larger cell mass ?
j) Does the integration of a more physiological tissue macroenvironment by the hollow fiber membranes into a growing cell mass result in a genotypic/phenotypic stabilization of proliferating human embryonic stem cells ?
k) Can a compartmentalized co-culture of feeder cells and human embryonic stem cells better support HESC in a growing cell mass ?
l) Can we maintain the stability of human embryonic stem cells *in vitro*, while they are proliferating as a larger mass ?

For several topics, experimental animal source or biopsied human tissue, as well as conventional petridish *in vitro* culture methods seem to provide appropriate tools for investigation. However, investigations focusing on the impact of exogenous factors could benefit from the use of purpose-built bioreactors that enable 3D high-density tissue co-culture. There is a considerable need for such *in vitro* stem cell systems, since the stem cell-derived tissues must be capable of stable and long-term integration ; into a bioreactor or, after transplantation, into existing physiological tissues, at least until they are replaced by the body's own tissue repair process, or permanently if self-repair is not possible (66). At least some of such studies require reproducible and controllable *in vitro* conditions.

We believe that to guide tissue assembly by the cells themselves, maintenance, and proliferation/differentiation of stem cells, a switch from the conventional two- or three-compartment systems towards a four-compartment technology is necessary.

Our working hypothesis states that 3D cell-cell contact of various stem cell populations in a perfused macroenvironment, providing decentralized mass exchange at physiological tissue gradients and integral oxygenation, will allow a better approximation to the in vivo situation. There, embryonic stem cells form microvascular structures and adult stem cells reside and act in such microvascularised tissues. A four-compartment bioreactor technology platform allows addressing the technical realization. Furthermore we hypothesize that stem cells themselves can create their own typical microenvironment in such *in vitro* culture models, and adult stem cells benefit from parenchymal/non-parenchymal cell co-culture in such systems for the creation of an organo-typical microenvironment.

Bioreactor Technology Overview

In vitro control of adult liver stem cells differentiation by mediators is studied by many groups. Of the progenitors, the "small hepatocytes/oval cells" were described more in detail (67,68,69). The effects of growth factors, cytokines or mediators and their role on growth/differentiation of fetal liver stem cells, as well as establishing of cell lines was also studied (70). Hepatocyte growth factor (HGF), for example, is generally known to stimulate hepatocytes to replicate *in vitro*, or oncostatin M, a multifunctional cytokine of the interleukin-6 family, has

Table 3. — **Culture Models for *In Vitro* Cell Growth and Maintenance (Hout & Gerlach)**

Technology	Cell Type	Citation
Cell entrapment within calcium alginate	Rat hepatocytes	Miura *et al.*, 1986 (92), 1990 (93)
Collagen sandwich culture	Rat hepatocytes	Dunn *et al.*, 1989 (94)
Collagen gel immobilization, perfusable culture	Human hepatocytes	Koebe *et al.*, 1994 (95-96)
Hollow fiber-based bioartificial liver with integral oxygenation (Gerlach CellModule)	Porcine liver cells	Gerlach *et al.*, 1994 (97-98), 1995 (99)
Micropatterned borosilicate wafers	Rat hepatocytes and 3T3 fibroblasts	Bhatia *et al.*, 1997 (100)

been shown to both induce maturation of mice fetal hepatocytes *in vitro* (71,72,73), and to attenuate fetal liver hematopoiesis (74).

Several authors noted that 3D culture is an important factor not only in accommodating an organ-like mass but also in enhancing physical cell-to-cell contact, accumulation of extracellular matrices, and local growth factor delivery, resulting (for example) in better maintenance of fetal mouse hepatocytes compared to those in monolayer cultures (75,76).

Aim of advanced bioreactor technology development for mamalian tissue culture is to allow cells to re-structure functional tissue with organ functions *ex vivo*. Additionally, bioreactor technology developers aim to further the understanding of the behavior of cells and the mechanisms of tissue formation. Many different bioreactor constructions have been developed and tested *in vitro* (Table 3).

It was pointed out by several authors that 3D culture is important not only in accommodating an organ-like mass but also in enhancing physical cell-to cell contact, accumulation of extra cellular matrices, and local growth factor delivery, resulting in much higher biochemical effects on the maturation of fetal mouse hepatocytes then those in monolayer cultures (77,78).

The general aim of bioreactor technology development for primary- and stem cells is the understanding of modeling of cells to allow recovery from isolation injury in vitro and to re-structure functional tissue with organ functions ex vivo. Furthermore, bioreactor technology aims to contribute to further the understanding of the behavior of cells, and to control mechanisms of tissue formation. This implies to engineer technology to create functional tissue in vitro. Several technologies have been developed (Table 4).

The development of specific 3D tissue density bioreactor technology for stem cells and co-cultures appears important in the context of the developiment of culture models and specific cells, as well as for subsequent translation of the findings to work on animal models, establish cell banks and possible clinical trials. Only few data are available about theoretical considerations in the development of such bioreactors.

Current bioreactor designs span a wide range of geometries, flow arrangements, and exchange conditions. Selected bioreactor motifs (e.g., multi-axial hollow fiber (79) and flat plate bioreactors (80)) have been modeled using simple engineering equations that relate operating parameters to conditions within the local cellular environment (e.g., plasma flow rates in cell chambers, oxygen tension profiles, etc.) and specific biological performance measures (e.g., detoxification, albumin production, etc.). These idealized geometries are excellent tools for investigating the impact of the microenvironment upon cell behavior and function ; however, the extension of these designs toward clinical dimensions has been a challenge. In contrast, the four-compartment hollow fiber-based bioreactor technology presented here has been scaled up from small analytical devices (cell mass of 0.1 gm) towards clinical bioartificial liver support units (cell mass of 600 gm).

The concept of Modular Extracorporeal Liver Support - MELS –

An extracorporeal liver support system has to support or substitute the main functions of the liver, providing detoxification, synthesis and regulation. To date, developments concentrate on either artificial detoxification systems (such as albumin dialysis (81) or adsorber suspension with activated charcoal particles and ion exchange resin (82,83,84)), or on biologic systems (like whole organ perfusion and bioreactors with liver cells (85)).

For detoxification (e.g. removal of bilirubin, bile acids, and toxins) simple, dialysis-like artificial detoxification systems have been shown to be efficient. The complex tasks of regulation (e.g. CNS transmitter precursors) and synthesis (e.g. coagulation factors) remain to be addressed by the use of human liver cells.

The Modular Extracorporeal Liver Support (MELS) concept combines different extracorporeal therapy units, tailored to suit the individual clinical needs of each patient (Fig. 4). The *CellModule* is a specific bioreactor charged with primary human liver cells, harvested from explanted livers found to be unsuitable for transplantation. The *DetoxModule* enables albumin-dialysis for the removal of albumin-bound toxins. The *DialysisModule* for continuous veno-venuous hemofiltration can be added to the system if required.

The mobile trolley-mounted liver support system consists of a blood circuit with a continuous plasma separation unit (e.g. CRRT, B. Braun, Melsungen, Germany), a high-flux dialysis filter (Fresenius Medical

Table 4. — **Bioreactor Constructions Tested *In Vitro* (Hout & Gerlach)**

Technology	Cell Type	Citation
Early perfusion chambers	Chick heart fibroblasts, human malignant epithelial cells, Chinese hamster cells, hybridomas	Christiansen *et al.*, 1953 (103); Rose, 1954 (104); Freed, 1963 (105); Katinger, 1985 (106)
Commercially available perfusion chambers	Bone marrow-derived osteoblasts	Minucells (107)
Commercially available systems for non-adherent cells	Hybridomas	VectraCell gas-permeable bags (108), Rotary Cell Culture System (109), Wave Bioreactor (110), CELLine (111), miniPERM Bioreactor (112), CellMax (113); Tecnomouse (114)
Commercially available system for bone marrow expansion	Hematopoetic stem cells	AastromReplicell (115)
Hollow fiber-based bioreactors	Mouse fibroblasts, human choriocarcinoma cells, Reuber hepatoma cells, human hepatocytes	Knazek *et al.*, 1972 (116); Wolf *et al.*, 1975 (117); Hager *et al.*, 1978 (118), 1983 (119)
Hollow fiber-based bioreactor with integral oxygenation	Human leukemic cell lines	Gloeckner *et al.*, 2001 (120)
Coaxial hollow fiber-based bioreactor with integral oxygenation	Rat hepatocytes	Macdonald *et al.*, 2001 (121)
Hollow fiber-based bioartificial liver with integral oxygenation (Gerlach CellModule)	Porcine and human liver cells	Gerlach *et al.*, 1994 (122), 1996 (123), 2003 (124-125); Sauer *et al.*, 2002 (126)
Flat sheet and hollow fiber-based bioartificial liver with integral oxygenation	Porcine hepatocytes	Flendrig *et al.*, 1997 (127)
Hollow fiber-based renal tubule assist device (Humes RAD)	Porcine renal tubule cells	Humes *et al.*, 1999 (128)
Flat membrane bioreactor with integral oxygenation	Porcine hepatocytes	De Bartolo *et al.*, 2000 (129)
Flat plate bioartificial liver with integral oxygenation	Porcine hepatocytes	Shito *et al.*, 2001 (130)
Micropatterned borosilicate wafers	Rat hepatocytes and 3T3 fibroblasts	Bhatia *et al.*, 1997 (131)
Biodegradable polymer bioreactor constructed via 3D printing	Rat liver cells	Kim *et al.*, 1998 (132)
Microfabricated bioreactor constructed via ion etching of silicon wafers	Rat hepatocytes	Powers *et al.*, 2002 (133-134)
Titanium mesh bioreactor	Rat bone marrow stromal osteoblasts	Bancroft *et al.*, 2003 (135)
Bioreactor containing hydrated polyester fibers and porcine autologous biomatrix	Porcine hepatocytes (10^{10})	Ambrosino *et al.*, 2002 (136)
Bioreactor containing nonwoven polyurethane matrix with integral oxygenation	Rat or pig hepatocytes	Linti *et al.*, 2002 (137)

Care, Bad Homburg, Germany), and a second circuit for plasma perfusion of the bioreactor (800 ml-bioreactor, Hybrid Organ, Berlin, Germany). The simple set-up allows the integration of any other standard renal replacement therapy device. Venous access is gained by placing a double-lumen dialysis catheter into either the internal jugular or the femoral vein. Blood is pumped through a hollow fiber plasma filter (Plasmaselect 0.4, Braun) at a rate of 150-250 mL/min. If necessary, a continuous infusion of heparin was performed for anticoagulation in order to achieve an activated clotting time of 160-180 seconds (ACT, Fresenius, Oberursel, Germany). For continuous exchange with the *CellModule*, the bioreactor is connected to the plasma circuit in counter-directional flow mode at 150-200 mL/min. The total extracorporeal volume is approximately 110 ml in the blood circuit, and 900 ml of plasma in the bioreactor and associated circuitry.

The *CellModule* is the multi-compartment bioreactor for extracorporeal liver support therapy, described above. In our actual concept, the *CellModule* is loaded with human liver cells, harvested from organs explanted for LTx but subsequently discarded due to steatosis, cirrhosis or mechanical injury. Cells are obtained through a 5-step collagenase liver perfusion (86). Under conditions of capillary perfusion, parenchymal and non-parenchymal cells form tissue by spontaneous organization to cellular aggregates, immobilized to the surface of the capillaries. Within these aggregates, channels are formed, representing neo-sinusoidal structures with a

reformation of a neo-space of Dissé. The cells produce their own biomatrix - the use of foetal calf serum and additional (animal derived) biomatrix collagen can be avoided. A cell mass of 400 g-600 g enables the clinical application of a liver lobe equivalent to a hybrid organ. The bioreactor constructions were investigated in vitro (87) and in vivo (88). The results are summarized above.

During the stand-by phase of 21 days (mean) prior to therapeutic use, the bioreactors are characterized routinely concerning metabolic activity on a daily basis and contamination can be excluded.

In liver failure the insufficient metabolism of endogenous toxins has been shown to be fatal. Most of these toxins are albumin-bound (1). In previous reports, liver assist devices based on albumin-dialysis were found to eliminate these toxins (89,90). The *DetoxModule* enables albumin-dialysis via a standard high-flux dialysis cartridge. Single-pass albumin dialysis (SPAD) is a simple implementation of albumin dialysis using standard renal replacement therapy machines (91) : The patient's blood flows through a circuit with a high-flux hollow fiber hemodiafilter (Fresenius HdF 100S polysulfone high-flux haemodiafilter, Fresenius AG, Bad Homburg). The other side of this membrane is cleansed by an albumin solution in counter-directional flow and discarded after passing the filter. One liter of a 4.5-liter bag with standard bicarbonate buffered dialysis solution is replaced by 1000 ml of 20% human albumin solution, resulting in 4.44% albumin solution. During therapy, the blood pump speed is adjusted to 130-180 ml/min ; the dialysis-pump speed is 600 ml/h.

30-75% of all patients in ALF show renal failure with fluid overload, electrolyte derangement and high levels of creatinin. Therefore, the MELS concept facilitates an integrated renal replacement therapy in terms of a continuous veno-venous hemodiafiltration via the high-flux hollow fiber hemodiafilter as part of the *DetoxModule*. A standard buffered aqueous solution is used (added after the filter, "postdilution") with a flow rate of 1000-3000 ml/h.

The proposed system for extracorporeal liver support is modular and combines cell based therapy with different forms of dialysis and detoxification. One modular system for all relevant extracorporeal liver support therapies facilitates handling on the intensive care unit and reduces costs for disposables, service, and technical support. Modularity allows the clinician to adapt the extracorporeal liver support to the individual, actual needs of the patient suffering from liver failure.

Reduction of albumin-bound toxins or drugs, reduction of the biochemical burden prior to liver transplantation, or reduction of bilirubin levels in cases of pruritic hyperbilirubinemia are easily treated with albumin dialysis. In severe cases of liver failure with hepatic encephalopathy, the *CellModule* is added. The additional *DialysisModule* facilitates the support of the failing kidneys in hepato-renal syndrome. Therapy can be started with the *DetoxModule* and the *DialysisModule* in every intensive care unit before the more sophisticated and logistically more demanding *CellModule* is added.

A liver support system has to provide detoxification (e.g. toxins, ammonia, bilirubin, endotoxins), synthesis (e.g. albumin, amino acids, coagulation factors), and regulation (e.g. acid-base-status, electrolytes, amino acids, CNS energy supply, CNS transmitter precursors). In this modular approach we consider the principal task of the *CellModule* in regulation and synthesis, secondary in detoxification. The *DetoxModule* enables detoxification in terms of removal of toxins from the patient's blood, as well as the reduction of the biochemical burden of the cells inside the *CellModule*. In addition, it replaces the bile excretion of the cells inside the bioreactor. The *DialysisModule* as renal replacement therapy permits regulation and detoxification.

The proposed concept is currently under clinical investigation in a phase I study.

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