Cytotechnology (2006) 50:163–179
DOI 10.1007/s10616-006-6336-4

Present and future developments in hepatic tissue engineering for liver support systems

State of the art and future developments of hepatic cell culture techniques for the use in liver support systems

Sonja Diekmann, Augustinus Bader and Stephanie Schmitmeier*
*Center for Biotechnology and Biomedicine, Cell Techniques and Applied Stem Cell Biotechnology, University of Leipzig, Deutscher Platz 5, 04103 Leipzig, Germany; *Author for correspondence (e-mail: stephanie.schmitmeier@bbz.uni-leipzig.de; phone: +49-341-97-31354; fax: +49-341-97-31359)*

Received 3 January 2006; accepted in revised form 3 January 2006

Key words: Bioartificial liver, Hepatocytes, Liver failure, Liver support systems

Abstract

The liver is the most important organ for the biotransformation of xenobiotics, and the failure to treat acute or acute-on-chronic liver failure causes high mortality rates in affected patients. Due to the lack of donor livers and the limited possibility of the clinical management there has been growing interest in the development of extracorporeal liver support systems as a bridge to liver transplantation or to support recovery during hepatic failure. Earlier attempts to provide liver support comprised non-biological therapies based on the use of conventional detoxification procedures, such as filtration and dialysis. These techniques, however, failed to meet the expected efficacy in terms of the overall survival rate due to the inadequate support of several essential liver-specific functions. For this reason, several bioartificial liver support systems using isolated viable hepatocytes have been constructed to improve the outcome of treatment for patients with fulminant liver failure by delivering essential hepatic functions. However, controlled trials (phase I/II) with these systems have shown no significant survival benefits despite the systems' contribution to improvements in clinical and biochemical parameters. For the development of improved liver support systems, critical issues, such as the cell source and culture conditions for the long-term maintenance of liver-specific functions *in vitro*, are reviewed in this article. We also discuss aspects concerning the performance, biotolerance and logistics of the selected bioartificial liver support systems that have been or are currently being preclinically and clinically evaluated.

Introduction

The liver as the central metabolic organ is responsible for many physiological functions, including detoxification and biotransformation. Therefore, acute liver failure often compromises multiple organs leading to the death of affected patients (Muto et al. 1988; Hughes et al. 1998). Despite the intensive medical care, the mortality of hepatic failure is still high, and its clinical management, therefore, remains a challenge (Rahman and Hodgson 2001). One of the main characteristics of the liver is its capacity to regenerate. In acute hepatic failure, the liver often retains its

regenerative ability. By contrast, this feature is hardly observed, if at all, in chronic liver failure (Koniaris et al. 2003; Black et al. 2004). In this case, the transplantation is the only life extending method. However, liver transplantation is beset by a scarcity of donor livers and a lack of immediate availability (Lee 1993; van de Kerkhove et al. 2004). In order to circumvent this problem, alternative methods have been developed to stabilize the conditions of patients with hepatic failure until the regeneration of the liver or availability of the donor organ (Rifai et al. 2003). Several extracorporeal detoxification systems, such as hemodialysis, hemofiltration, adsorption, plasma exchange, and plasma perfusion, to support liver functions have been clinically tested with varying success (McLaughlin et al. 1999). Since the liver commands a myriad of functions, including hormonal regulation, biotransformation and detoxification, protein synthesis, lipid and glucose metabolism, production of bile components, as well as pH regulation (Kmieć 2001), complex biochemical pathways exist that cannot be simply replaced by these non-biological liver support systems. From a medical point of view, the purely detoxifying and filtrating capacity of anorganic devices has been a relevant addition to the treatment regimens. Apart from charcoal- and polymer-based regimens, the use of albumin as a carrier and shuttle molecule was also proven to be a successful treatment option, which is currently in widespread use, such as the molecular adsorbent recirculation system (MARS) (Mitzner et al. 2001) and the more recent addition to the field, namely the Prometheus system (Evenepoel et al. 2005). Both systems replace the detoxification function of the liver by removing of a number of toxins. Comparison of these systems has shown that Prometheus treatment resulted in significant better reduction ratios of bilirubin, ammonia and urea (Krisper et al. 2005). However, both systems cannot reflect the full synthetic, metabolic and regulatory functions of the liver parenchyma. For this reason, new bioartificial systems have been developed that contrary to the classic systems include a cellular hepatic component. These biological systems are believed to be more likely to provide many essential hepatic functions, namely detoxification, metabolism, and biosynthesis, than the techniques based on the non-biological liver support.

Anatomy of the liver

In the liver, parenchymal hepatocytes are in functional contact with non-parenchymal cells, including bile duct cells, sinusoidal endothelial cells, Kupffer cells, stellate cells (fat storing Ito cells) and Pit cells (Figure 1). The assembly of the hepatic sinusoidal cells points to the functional interaction between parenchymal and non-parenchymal cells. Sinusoidal endothelial cells differ from other endothelial cells by the lack of a subjacent basal lamina (De Leeuw et al. 1990; Wake 1999). These cells have a scavenger function by eliminating macromolecules through endocytosis (Elvevold et al. 2004; Enomoto et al. 2004). They form fenestrae to filter fluids, dissolved substances and particles from serum which are then adsorbed and metabolized by hepatocytes. Bile canaliculi are located on the lateral surface of adjoining hepatocytes. Both parenchymal and non-parenchymal cells are embedded in an extracellular matrix consisting of collagen I, III, IV and V, laminin, fibronectin, tenascin, nidogen and the glycoprotein SPARC (Secreted Protein Acidic and Rich in Cysteine) (Martinez-Hernandez and Amenta 1993).

Function of hepatocytes and requirements on bioartificial liver support systems

Most of the biochemical functions are carried out by mature hepatocytes (Kmieć 2001). Fulminant

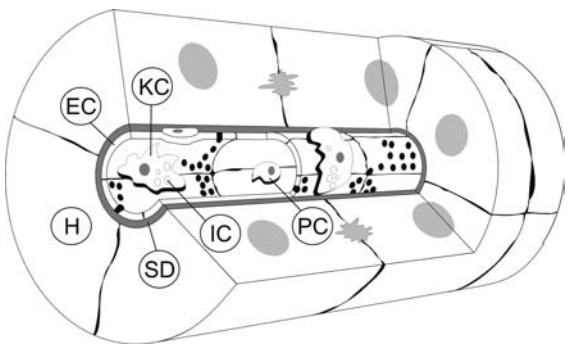

Figure 1. Schematic drawing of liver tissue. The liver consists of differentiated hepatocytes (H) separated from fenestrated endothelial cells (EC) by the Space of Disse (SD). Black filled circles in the endothelium depict fenestrae. Stellate or Ito cells are fat-storing cells (IC). Kupffer cells (KC) function as liver-specific macrophages, while Pit cells (PC) are a type of natural killer cells (drawn by C. Mohr).

hepatic failure is a consequence of the loss of normal liver function and often occurs as a result of autoimmune and viral hepatitis, hepatocellular cancer, exposure to toxins such as alcohol and drugs, or trauma (Gimson 1996). The concept of a bioartificial liver support system is based on the assumption that only hepatocytes perform a wide range of liver-specific functions, and it uses primary hepatocytes or a hepatoma cell line due to their expression of highly differentiated functions (Sauer et al. 2001). For the development of such a liver support system, special attention has been paid to providing the architectural basis for the reconstruction of a proper cellular microenvironment that ensures the highest and prolonged functional activity of the hepatocytes. The main problem of such systems is their biotolerance in terms of the duration of the treatment because of the possible immune reaction provoked by a direct contact between animal (xenogeneic) hepatocytes and the recipient's blood or plasma as observed with porcine liver cells (Hasegawa et al. 1999). Therefore, it is crucial for successful long-term stable hepatocyte cultivation in a bioartificial system that the cells are protected from the host immune system. Additionally, adequate oxygenation and nutrient supply are particularly critical factors in terms of maintaining hepatocyte viability and function (McClelland et al. 2003). These parameters have been improved to provide hepatocytes an *in vivo*-like environment in bioartificial liver support systems (Gerlach 1996; De Bartolo and Bader 2001). Furthermore, logistical aspects such as storage, transportation and scale-up of the systems have still to be optimized to offer flexible and independent devices for the effective clinical application.

Cell source

Biological systems have focused in the past on maintaining xenogeneic, allogeneic primary hepatocytes, or human cell lines alive within the respective liver support systems. On the basis of results from human liver resections, at least 20% of the liver mass may be required for adequate liver support (Morsiani et al. 2002a). According to a theoretical calculation, an adequate mass of up to at least $1–2 \times 10^{10}$ viable hepatocytes is necessary to maintain normal human liver function and is needed in a liver support system to treat patients with liver failure (Morsiani et al. 2002a; van de Kerkhove et al. 2005b). The methods used for isolation of primary human hepatocytes are less than optimal because of the limited number of donor organs (Dou et al. 1992; Runge et al. 2000). Therefore, other cell sources, including animal (xenogeneic) hepatocytes, hepatoblastoma cell lines, and immortalized hepatocytes have been used for their application in bioartificial liver support systems (Tsiaoussis et al. 2001). However, a concern with the use of hepatic tumor cell lines, e.g. HepG2, is the possible risk in the transmission of potentially tumorigenic cells to patients (Louha et al. 1997). Instead, primary human and xenogeneic hepatocytes are used in bioartificial systems which have been or are currently in various stages of clinical evaluation. Gerlach et al. have used human hepatocytes obtained from donor organs that are unsuitable for liver transplantations (Gerlach et al. 2003). However, due to a lack of human organ availability the current main source of hepatocytes for bioartificial systems is xenogeneic material. Primary porcine hepatocytes have preferably been used as the xenograft candidates regarding differentiated metabolic functions and high-yield retrieval (Sielaff et al. 1995; te Velde et al. 1995; Gregory et al. 2000). Preparation of hepatocytes from pig liver has been shown to deliver a sufficient amount of cells for a bioartificial system (De Bartolo and Bader 2001). However, clinical application of xenogeneic hepatocytes has become a controversial issue with regard to xenotransplantation-associated problems. Porcine xenografts, for example, elicit a severe humoral and cellular immunologic response in humans due to the presence of the carbohydrate Galα (1–3)Gal epitope on pig cells, thereby compromising the functionality of the bioartificial liver (Baquerizo et al. 1999; van de Kerkhove et al. 2005a). Besides these immunological problems, proteins released by porcine hepatocytes do not carry out the same functions as their human counterparts. Another possible risk is the transfer of viral pathogens from the xenograft donor to the recipient (Fishman and Patience 2004). To date, several potentially pathogen viruses have been identified, among which are porcine endogenous retrovirus (PERV), porcine cytomegalovirus (PCMV), and porcine lymphotropic herpesvirus (PLHV). In order to prevent a viral infection in

patients, hepatocytes from SPF (specific pathogen free) animals which are raised under strictly controlled conditions may probably be one of the best xenograft candidates (Sauer et al. 2003). However, since PERV genomes are present in all porcine cells (Blusch et al. 2002), transmission of this virus from SPF animals to recipients cannot be excluded. Interestingly, PERV transmission into humans has never been observed *in vivo* (Pitkin and Mullon 1999; Irgang et al. 2003). Nevertheless, further interest has developed in finding new cell sources for bioartificial support systems, as well as for transplantations. Human liver cells, that have been immortalized using different systems, have been suggested as an interesting cell source. The immortalized human cell line HepZ, for example, was obtained by transfecting the cells from human liver biopsy with the plasmids containing the albumin-promotor-regulated antisense constructs against the negative controlling cell cycle proteins Rb and p53 (Werner et al. 1999). The cells were co-transfected with plasmids harboring genes coding for the cellular transcription factor E2F and D1 cyclin to overcome the G1-restriction point. Moreover, Kobayashi et al. have established the Cre-loxP recombination system that targets cells in their final differentiated state (Kobayashi et al. 2003). This system uses a retroviral vector expressing the immortalizing gene simian virus 40 large T-antigen (SV40Tag) which, in turn, is located between loxP sequences (consensus 34 bp DNA recognition sites). This gene can be removed by the Cre recombinase-mediated reaction (Cre-loxP reaction) (Nagy 2000). These and other so far established immortalized human hepatocyte cell lines have been shown to vary in their tumorigenicity and hepatic functions (Hoekstra and Chamuleau 2002). Although immortalized cells have the capacity to be both highly proliferative and differentiated, they tend to lose critical functions, e.g. drug and ammonia metabolism, *in vitro* compared with primary counterparts, thereby limiting their application in the support system (Cascio 2001). Furthermore, comparison of primary human hepatocytes and the hepatoma cell line (HepG2) has shown that the former are more suitable for the development of liver support systems with respect to their biotransfomation properties (Wilkening et al. 2003). Expansion of autologous hepatocytes in patients with liver disease is not advantageous for at least the following five reasons: (i) The underlying disease, i.e. liver carcinoma or hepatitis, could be transmitted to the liver support system; (ii) Liver failure is an acute phenomenon, and creating a liver mass of several grams *in vitro* would require plenty of time; (iii) Removing healthy liver tissue from an impaired patient in a critical situation could further endanger the patient's life. The liver has a tendency to compensate parenchymal loss until the utmost limit is reached. A patient with liver failure would not tolerate well further removal of liver mass even for starting a cell culture process; (iv) Autologous or allogeneic hepatic adult stem cells, or precursor cells could serve as an alternative cell source, but it will be necessary to differentiate them into functional mature hepatocytes *in vitro* and cultivate them in the relevant number ($\sim$1–2 $\times$ 10^{10} cells) required for a liver support system without a loss of differentiation potential. No data on the successful *ex vivo* expansion of stem cells have been reported elsewhere in the literature. Furthermore, factors, such as medium formulations, supplementation of certain growth factors, as well as the microenvironment (He et al. 2003; Lowes et al. 2003; Semino et al. 2003; Suzuki et al. 2003), are decisive for the differentiation of adult stem cells into mature hepatocytes *in vitro*, and are accompanied by the high cost; (v) In contrast to the adult stem cells, foetal hepatocytes have high mitotic rates independent of endogenous stimuli and therefore could be an ideal cell source for allogeneic liver cells in the future. However, the risk of tumorigenicity, incomplete differentiation and ethic concerns may limit their clinical application in the next years.

Maintenance of liver-specific functions *in vitro* and cell proliferation

Maintaining differentiated hepatocyte functions *in vitro*, in particular in extracorporeal liver support systems, remains a challenge. The sufficient conditions necessary for long-term stability of hepatocyte functions are still being optimized. Hepatocytes lose their metabolic activities within a short period of time *in vitro* due to the deprivation of their original architecture and polarity (Nyberg et al. 1992b). The state of hepatocytes can be modulated by factors, such as cytokines and

cellular contacts, as well as by the extracellular matrix (Isom et al. 1985; Ben-Ze'ev et al. 1988). Since a complex extracellular matrix is necessary to maintain long-term differentiated hepatocytes *in vitro* there has been greater interest in developing effective three-dimensional systems that mimic the *in vivo* environment. It is well known that the extracellular matrix modulates the expression of liver-specific genes, including that of albumin, cytochromes P450, and transferrin (Schuetz et al. 1988; Saad et al. 1993). In contrast to a single layer of hydrated rat tail tendon collagen gel, the sandwiching of rat hepatocytes between two collagen gel layers preserves a variety of liver-specific functions (Dunn et al. 1991, 1992). A recent study using rat hepatocytes has demonstrated that ECM remodeling in response to cytokines induces cell proliferation, an important parameter of liver regeneration (Serandour et al. 2005). *In vivo*, these cytokines are produced by non-parenchymal hepatic cells in the regenerative process after hepatic injury or hepatectomy (Michalopoulos and DeFrances 1997; Ramadori and Armbrust 2001). This process requires proliferation of hepatocytes and non-parenchymal hepatic cells to restore damaged liver-specific functions and/or the liver mass (Kang et al. 2004). A cross-talk between these two cell types is not only important for cell proliferation but also for the maintenance of the differentiated stage of hepatocytes. This is perhaps one of the reasons why hepatocytes in co-cultures exhibit elevated metabolic activities over a long period of time compared to those in single cultures (Auth et al. 1998). Proliferation of hepatocytes is initiated by cytokine-mediated G_0/G_1-transition of the cells, while the G_1/S-transition is also controlled by hormones (Costa et al. 2003; Taub 2004). Due to the involvement of certain cytokines and hormones in controlling the state of hepatocytes, the use of these components as medium supplements for the *in vitro* hepatocyte cultivation is an important aspect. A further aspect in liver regeneration after partial hepatectomy is the shear stress (Sato et al. 1999; Braet et al. 2004). A possible relation between elevated blood flow associated with partial hepatectomy has been suggested. However, the influence of the shear stress on triggering further events, leading to hepatocyte proliferation, has only been proven *in vivo* (Schoen et al. 2001).

Bioartificial liver support systems

A large number of liver support systems have been developed to promote cell organization with the aim of providing *in vivo* conditions. These systems include the flat membrane configuration, arrangement of membranes in fibers (hollow fiber system), the encapsulation technology and cell aggregates, and they vary greatly with respect to their microenvironment. Table 1 summarizes the bioreactor designs that have been proposed and studied.

Flat membrane systems

Cultivation of isolated hepatocytes on a single gel has been shown to be ineffective since the cells lose their metabolic abilities within a short period in culture. Three-dimensional cell adhesion cultures on an extracellular matrix provide an alternative technique (Bucher et al. 1990). Among the first cultivation systems were cultures with rat hepatocytes entrapped in collagen gel (a sandwich configuration) or on a reconstituted basement membrane gel (Schuetz et al. 1988; Dunn et al. 1989). The flat membrane bioreactor allows a high-density hepatocyte culture under sufficient oxygenation conditions closely corresponding to the *in vivo* microenvironment (De Bartolo and Bader 2001). In this system porcine hepatocytes are co-cultured with non-parenchymal hepatic cells within an extracellular matrix between oxygen-permeable flat-sheet polymeric membranes as individual plates, thereby enabling the cells to remain polarized *in vitro* and maintaining constant liver-specific functions. A microporous polytetrafluoroethylene membrane that separates the cell compartment from the medium compartment protects the cells from shear forces and controls transfer of metabolites during continuous flow (Figure 2). One of the prerequisites we have demanded for the flat membrane bioreactor, was thorough biochemical testing. In this context, our studies have shown that *in vivo*-like quantitative as well as qualitative performance could be achieved *in vitro* using pharmaceutical drugs as test candidates (Bader et al. 1992, 1996, 1998; Langsch and Bader 2001). Currently, rat and human hepatocytes are being successfully cultured on novel modified polyetheretherketone membranes

Table 1. Summary of the bioartificial liver support designs that are described in this article.

Bioartificial liver support designs	Features	Cell source	Max. functional cultivation time	Advantages/Disadvantages	Preclinical/Clinical test	References
Flat membrane system	Cell cultivation in sandwich configuration or in a flat configuration on membranes Bioreactor with stacked collagen gel sandwich culture	Rat Pig Human	< 1 month	*Pros*: Uniform cell distribution *In vivo*-like microenviroment Ease of scale-up Cryopreservation *Cons*: Potential large dead volume Low surface area-to-volume ratio Limited protection against viral infection in humans by xenogeneic cells Scale-up for logistics	Preclinical rat and pig	Bader et al. (1998) De Bartolo et al. (2000, 2004, 2005) Langsch and Bader (2001) Shito et al. (2003) Fruhauf et al. (2004)
Hollow fiber system	Cell cultivation in intra-or extrafiber space	Rat Pig	< 25 days	*Pros*: Immunoisolation Protection against viral infection in humans by xenogeneic cells In *vivo*-like microenvironment Protection from shear stress Ease of scale-up	Preclinical pig, rabbit, rat	Nyberg et al. (1992a, c, 1993, 1996, 1999) Jauregui et al. (1994, 1995) Gerlach (1996) Ellis et al. (1996) Flendrig et al. (1997, 1999) Sauer et al. (2001)
	Spirally-wound fabric scaffold and integrated hollow fiber oxygenation			*Cons*: Non-uniformed cell distribution Limited cryopreservation Limited mass transfer and total diffusion surface area for cells	Clinical BLSS, LSS/MELS, ELAD, HepatAssist AMC-BAL, RFB	Mazariegos et al. (2001) Pahernik et al. (2001) Jasmund et al. (2002) Morsiani et al. (2002b)

						Demetriou et al. (2004) van de Kerkhove et al. (2005b)
Encapsulation technology	Cells encapsulated in coated biomaterial beads	Rat Pig Hepatoblastoma	< 16 days	*Pros*: *In vivo*-like microenvironment High surface area to volume ratio Immunoisolation High capacity for cell mass Ease of scale-up Cryopreservation *Cons*: Risk of release of cells Limited mass transfer	Preclinical mice, UCLA-BAL (rat)	Babensee et al. (1992) Dixit et al. (1993), Dixit and Gitnick (1998) Joly et al. (1997) De Vos et al. (1997) Glicklis et al. (2000) Uludag et al. (2000) Legallais et al. (2000) Canaple et al. (2001) Orive et al. (2004) David et al. (2004) Mai et al. (2005)
Aggregates/Spheroids	Cell assembly to aggregates/spheroids on non-adherent substratum, roller bottles or in spinner flasks	Rat Pig	< 21 days	*Pros*: Cell-cell contact in colonies Ease of scale-up *Cons*: Oxygen and nutrition limitation (> 100 μm) Limited protection against viral infection in humans by xenogeneic cells Limited cryopreservation (depending on the solution used)	Preclinical PUF-HALSS (pig)	Koide et al. (1990) Sakai et al. (1996) Xu et al. (2003) Yamashita et al. (2003) Glicklis et al. (2004) Lee et al. (2004) Eschbach et al. (2005)

BLSS, bioartificial liver support system (Excorp Medical, Mineapolis, MN, USA); LSS/MELS, liver support system/modular extracorporeal liver support (Charité, Humboldt University, Berlin, Germany); ELAD, extracorporeal liver assist device (Amphioxus Cell Technology, Houston, TX, USA); HepatAssist (Circe Biomedical, Lexington, KY, USA); AMC-BAL, Academic Medical Center-bioartificial liver (University of Amsterdam,The Netherlands); RFB, radial flow bioreactor (University of Ferrara, Italy), UCLA-BAL, University of California at Los Angeles-BAL (USA), PUF-HALSS, polyurethane foam/spheroid culture-hybrid artificial liver support system (Kyushu University, Fukuoka, JP).

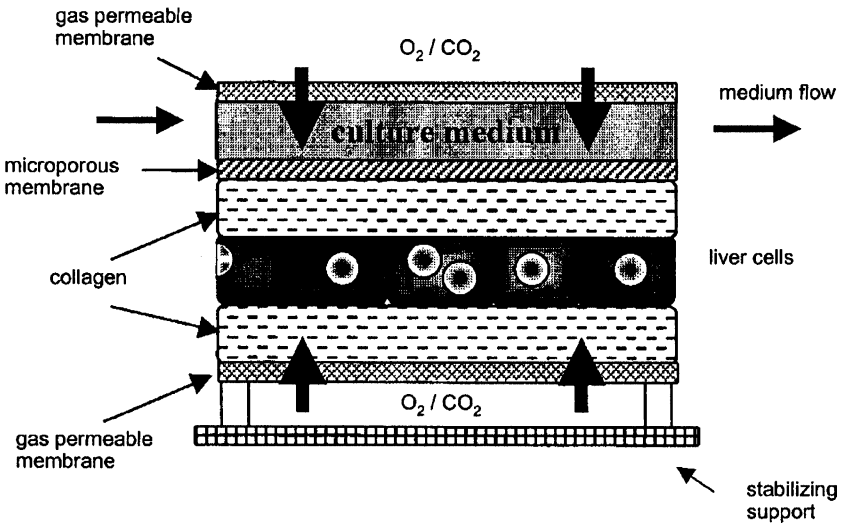

Figure 2. Schematic diagram of hepatocytes embedded in two layers of collagen in a flat configuration used in the flat membrane bioreactor (from De Bartolo et al. 2000).

(PEEK-WC and PEEK-WE-polyurethane) which have been proposed to be promising biomaterials in liver support systems (De Bartolo et al. 2004).

The use of a flat membrane bioreactor as an extracorporeal liver support system, however, is accompanied by some disadvantages, such as the potential large dead volume and the low surface area-to volume ratio, as well as providing limited protection against viral infection by xenogeneic cells (depending on the molecular weight cut-off of the membranes used). Nonetheless, improvements of these factors will allow its use in a clinical setting in the near future.

Hollow fiber systems

The development of hollow fiber technology allows for human or animal hepatocytes to become an integral part of a bioartificial liver support system and potentially increases clearance efficiency of an *ex vivo* method. The cells in hollow fibers are separated from blood or plasma by a semipermeable membrane with a defined molecular weight cut-off. The cylindrical form and small size of the hollow fibers limits the diffusion distance which separates the cells from the surrounding medium. Animal or human hepatocytes are cultured outside the lumen of fiber membranes (extrafiber space), while blood, plasma or culture medium is pumped through the fiber lumen (Sussman and Kelly 1993; Jauregui et al. 1994) (Figure 3a). In contrast, Nyberg et al. have suspended rat hepatocytes in a three-dimensional gel which was injected into the intrafiber space of hollow fibers in a perfused bioreactor (Nyberg et al. 1992c) (Figure 3b). To meet hepatocyte demands on their environment and their need for oxygen, a bioreactor incorporating design concepts of a hollow fiber oxygenator (OXY-HFB) was built (Jasmund et al. 2002). Alternatively, a bioreactor has been constructed that consists of a spirally wound, nonwoven polyester matrix in a cartridge for hepatocyte immobilization and aggregation, and of integrated hollow fibers for low metabolite gradients, decentralized oxygenation, and CO_2 removal (Flendrig et al. 1997). Medium or plasma is in direct contact with the hepatocytes by perfusion through semipermeable membranes. Hollow fiber membranes with different nominal molecular weight cut-offs (<400 kD) were tested for immunoprotection of xenogeneic hepatocytes and as viral barriers against PERV (Nyberg et al. 1992a, 1999). Studies on the use of polymeric semipermeable membranes, such as polycarbonate and cellulose acetate membranes, as well as of nonwoven polyurethane-based biomatrices in microfibers to support hepatocyte adhesion and metabolic functions, and to serve as immunoselective barriers in liver support systems,

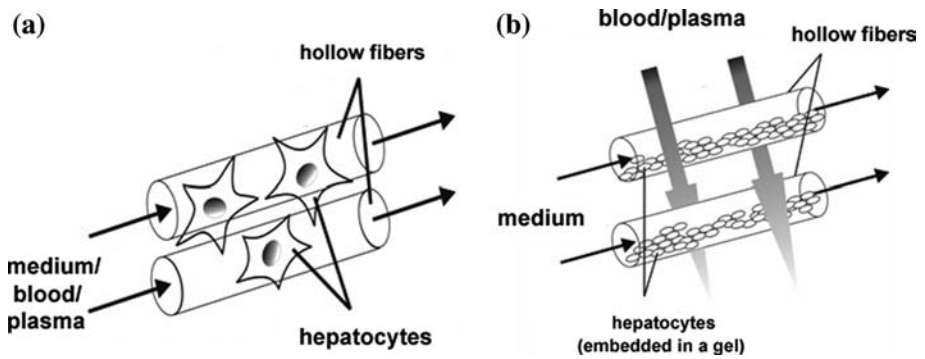

Figure 3. The diagram depicts two schemes of hollow fibers. (a) Hepatocytes are cultured on the extracapillary side of the semipermeable fibers while blood, plasma or medium flows through the lumen. The scheme is based on a system described by Sussman et al. (1992). (b) Hepatocytes are embedded in a gel injected into the intrafiber space of hollow fibers as described by Nyberg et al. (1992a). Blood or plasma perfuses between the hollow fibers, while medium flows through the fiber lumen.

seem to be promising (Pahernik et al. 2001; De Bartolo et al. 2002). Very recently, new polymeric semipermeable membranes used in biomedical devices for optimal detoxification and oxygenation of blood were the subject of investigation (Curcio et al. 2005). Although most capillary hollow fiber-based liver support designs provide an effective immunoprotection, they have some inherent physical limitations with respect to total diffusion surface area and capacity for hepatocyte mass.

Encapsulation technology

Direct contact of xenograft cells with plasma or blood is known to elicit a host immune response. Microencapsulation technology has, therefore, been developed to provide sufficient isolation of xenogeneic cells from the recipient's immune system within a support system. This technique is based on the encapsulation of xenogeneic hepatocytes with a biomaterial that allows nutrients, oxygen, and stimuli to cross the semipermeable material, while components of the immune system are excluded (Orive et al. 2003). Several biomaterials have been tested for their immune- and biocompatibility (Lacik et al. 1998; Honiger et al. 2000; Muraca et al. 2000; Quek et al. 2004). Polyanionic alginate, a polysaccharide with gel forming properties, is composed of mannuronic acid (M) and guluronic acid (G), and is commonly applied in combination with polycationic poly-L-lysine (PLL) for immunoprotection (Orive et al. 2004). As the biocompatibility (host inflammatory response by induction of the fibrotic reaction to capsules, porosity, stability, and PLL-binding of capsules) of alginate capsules strongly depends on the G/M-ratio of the alginate applied, the alginate was enzymatically modified to improve the biocompatibility of alginate/PLL microcapsules (King et al. 2003). It has been suggested that immune response is also reduced by applying alginates with a lower G-content and by introducing alginates with a high degree of purity (De Vos et al. 1997). Other systems of encapsulation involve the replacement of PLL with poly-L-ornithine (PLO), chitosan, or agarose, resulting in improved biocompatibility or in increased mechanical stability (Uludag et al. 2000; Orive et al. 2004). Entrapment of rat or pig hepatocytes within coated alginate beads enables the cells to maintain their long-term metabolic functions (Miura et al. 1988; Joly et al. 1997) (Figure 4). This technique used in liver support systems benefits from the presence of a three-dimensional hepatic environment in combination with an optimal volume-surface ratio of capsules which guarantees to meet hepatocyte demand for nutrients and oxygen (Legallais et al. 2000). Co-encapsulation of hepatocytes with non-parenchymal hepatic cells leads to the increased metabolic activity of hepatocytes, an observation which can be explained by the cytokine-release and ECM-production by non-parenchymal cells (Stange and Mitzner 1996). Improvements of the liver-specific functions can also be achieved by co-encapsulation of hepatocytes with ECM components (Babensee et al. 1992; Quek et al. 2004; Seo et al. 2005). Very recently, mouse hepatocytes

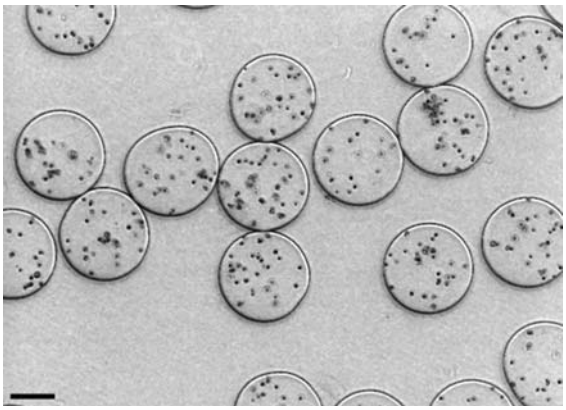

Figure 4. The phase contrast micrograph shows encapsulated primary porcine hepatocytes. The capsules were formed with alginate entrapping the cells and placed in culture medium for 1 day ($\times$ 100). The bar is equal to 200 μm (S. Diekmann, unpublished observation).

attached to xyloglucan, a synthetic extracellular matrix, were embedded in alginate capsules, demonstrating enhanced metabolic functions in such a three-dimensional space (Seo et al. 2005). Fremond et al. have shown that encapsulated hepatocytes in a bioreactor represent an effective model for bioartificial liver support systems (Fremond et al. 1993). The application of a fixed bed bioreactor is, however, associated with the problem of channels forming which prevent a uniform flow around the capsules, possibly inducing a high level of shear stress which can release the cells from the capsules (Dore and Legallais 1999). A more successful model is the current use of a dynamic (fluidized bed) bioreactor in which the diffusion coefficient of a compound tested is much higher than under batch (static) conditions (David et al. 2004).

Aggregate culture

Spherical aggregates (spheroids) of hepatocytes have been histologically shown to hold a three-dimensional structure with a bile canaliculus-like network. These cells resume cell-cell contacts by being prevented from attaching to the substratum, they retain many morphological *in vivo* hepatic characteristics, and thereby maintain viability and metabolic functions to a greater extent and a longer period of time than those in monolayer cultures (Tong et al. 1994). A range of methods has been developed for the generation of spheroids from animal hepatocytes, including non-adherent substratum, e.g. a neutral charge polymer poly(2-hydroxyethyl methacrylate (pHEMA) for self assembly, and spinner flasks (Koide et al. 1990; Sakai et al. 1996) (Figure 5). Hetero-spheroids of hepatocytes and non-parenchymal hepatic cells on a synthetic polymer were shown to further enhance long-term liver functions (Yamada et al. 2001). Moreover, Michalopoulos et al. have suggested using the hepatocyte organoid (composed of proliferating hepatocytes and non-parenchymal hepatic cells) culture technique that a combination of certain cytokines, such as hepatocyte growth factor (HGF) and epidermal growth factor (EGF), and dexamethasone plays a role in the formation and structure of the *in vivo*-like architecture of hepatocytes (Michalopoulos et al. 2001). Although hepatocytes in spheroids possess improved liver-specific functions and prolonged differentiated cell state *in vitro*, a concern is to be expressed regarding the size of the spheroids (Glicklis et al. 2004). Increasing spheroid size was found to induce cell necrosis due to the limited mass diffusion of oxygen and nutrients. A maximal level of oxygen consumption and albumin secretion by viable hepatocytes was reached in 100-μm diameter hepatic spheroids. More recently, a device composed of microstructured scaffolds was developed for the formation of small rat liver cell aggregates and was found to sustain hepatic metabolic functions for several days (Eschbach et al. 2005). Disadvantages

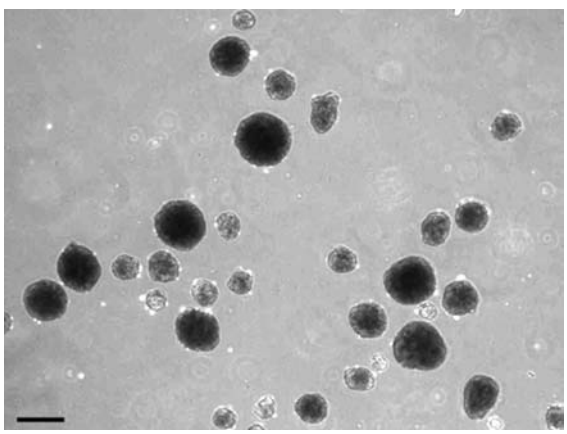

Figure 5. Primary mouse hepatic spheroids observed under an inverted light microscope after cultivation in a spinner flask for 5 days ($\times$ 50). The bar is equal to 100 μm (M. Funke, personal communication).

of the direct use of spheroids in a bioartificial liver system include the absence of an immunological isolation and the possible embolization of hepatocytes or non-parenchymal cells escaping from organoids into the system. Thus, hepatocyte aggregates are mostly immobilized in hollow fibers for the construction of a bioreactor (Lorenti et al. 2003; Gan et al. 2005).

Logistics of bioartificial liver support systems

Cryopreservation of hepatocytes allows the possible application of the cells for short-term support of patients with hepatic failure, it avoids the costs of long-term hepatocyte culture, and reduces the risk of contamination that exists during prolonged cell culture. However, cryopreservation of hepatocytes is associated with decreased cell functional activities and increased cell apoptosis (Guillouzo et al. 1999; Hengstler et al. 2000). It has been suggested that inhibition of proteins involved in apoptosis protects the cells from this process and preserve their viability after cryopreservation (Matsushita et al. 2003). Another method to reduce injury to hepatocytes during the cryopreservation step is to encapsulate the cells before cryopreservation (Dixit et al. 1993). These cells were shown to survive the procedure, maintaining their viability and metabolic functions (Canaple et al. 2001). While hepatocyte spheroids can be cryopreserved in defined solution (Lee et al. 2004), the use of the cryopreservation technique is limited in hollow fiber systems (unpublished observation). In contrast, flat membrane plates covered with a cryoprotective solution are completely cryopreservable (unpublished observation). All techniques discussed in this article have advantages in the large scale bioreactors for liver support systems due to their easy upscaling (Gerlach et al. 1993; Flendrig et al. 1997; De Bartolo and Bader 2001; Sauer et al. 2001). Systems using hepatic spheroids and encapsulated hepatocytes can easily be scaled to the cell mass needed to sustain the patient's life but may be associated with the possible dead volume and limitations of the mass transfer. Available space and flexible transportation are concerns which have arisen due to the unlimited upscaling of flat or stacked plate designs and hollow fiber devices.

Creation of a duty-service cell laboratory in specialized liver support centers should meet the frequency of treating patients with liver failure by providing ready-to-use liver support systems. The availability of such systems will improve the survival rate in patients by serving as a bridge to transplantation and to liver regeneration.

Liver support systems in preclinical and clinical test

The classical non-biological dialysis and filtration methods of liver support could decrease mortality in patients with moderate liver failure. However, these approaches have met with limited success due to their partial replacement of liver-specific functions, i.e. detoxification. Because of the diverse function of the liver, biological liver support systems have been constructed that rely on the functionality of hepatocytes from xenogeneic or human origin. Various liver support systems have been preclinically and clinically examined for their *in vivo* performance to date. In preclinical testing with liver support systems, significant improvement of survival time in hepatectomized animals or animals with moderate to severe liver failure could be achieved (Jauregui et al. 1995; Dixit and Gitnick 1998; Flendrig et al. 1999; Shito et al. 2003; Yamashita et al. 2003; Fruhauf et al. 2004). However, it is difficult to assess and compare the efficacy of these systems, in part because of the heterogeneity of the animal models used. Several liver support devices based mainly on the capillary hollow fiber system, have entered a Phase I/II clinical safety evaluation. Among them are the extracorporeal liver assist device (ELAD), the bioartificial liver support system (BLSS), the liver support system (LSS), the modular extracorporeal liver support (MELS), The Academic Medical Center bioartificial liver (AMC-BAL), the Hepat-Assist, and the radial flow bioreactor (RFB). The ELAD uses a human hepatoma cell line C3A grown in the extracapillary space of hollow fiber capillaries (Sussman et al. 1992). The patient's blood flows through the cartridge. Despite its high production of albumin and α-fetoprotein the C3A cells do not sufficiently express some specific functions such as ammonia detoxification and ureagenesis (Hoekstra and Chamuleau 2002). In a pilot-controlled clinical trial, the median period of the ELAD treatment was 72 h, but there was no significant difference in the survival rate between the ELAD-treated patients and the controls (Ellis

et al. 1996). The BLSS system uses primary porcine hepatocytes and perfuses whole blood through the bioreactor. The cells mixed with collagen are infused into the extracapillary space of fibers. Following the 12-h treatment, PERV transmission from the animal cells to patients was not observed (Mazariegos et al. 2001; Kuddus et al. 2002). Survival outcome has not been reported for this system. The MELS consists of the CellModule (the LSS), a multi-compartment bioreactor loaded with primary human hepatocytes, that are obtained from discarded donor livers, combined, if required, with a DetoxModule for albumin dialysis and a DialysisModule for continuous veno-venuous hemofiltration (Sauer and Gerlach 2002). The overall treatment time ranged between 7 and 144 h, and all patients survived until transplantation (Sauer et al. 2002). The AMC-BAL is based on a bioreactor with an integral oxygenator and a spirally wound matrix for small aggregates of primary porcine hepatocytes (van de Kerkhove et al. 2005b). The particular feature of this system is the direct contact of the patient's plasma with the cells, resulting in an optimal mass transfer and direct oxygenation. The maximal AMC-BAL treatment was 24 h with no PERV transmission from animal cells to the patients and a 100% survival rate in a small number of patients used in this study until transplantation. The HepatAssist system has been tested in the largest controlled clinical trial involving 171 enrolled patients (Demetriou et al. 2004). This liver support system is comprised of cryopreserved primary porcine hepatocytes in the extracapillary space of fibers of the bioreactor. The plasma of the patient flows through the capillary lumen. Treatment time was 6–8 h with no evidence of viral transmission from the porcine cells to the patients. Improved survival was only found in the liver support system-treated patients with fulminant/subfulminant hepatic failure compared with the controls. However, when considering the entire patient population enrolled in this study the difference in 30-day survival was not statistically significant. In the RFB system, primary porcine hepatocytes are entrapped within woven–nonwoven polyester fabric (Morsiani et al. 2001). Treatment lasted 6–24 h and was well tolerated in patients. PERV transmission was not detected during the short-term follow-up (Morsiani et al. 2002b). Amelioration of the neurologic status was only observed in patients during treatment with the HepatAssist, the AMC-BAL, the RFB, and the MELS system.

Kjaergard et al. conducted an analysis of the published clinical trials that used a variety of artificial and bioartificial liver support systems for acute and acute-on-chronic liver failure up to September 2002 (Kjaergard et al. 2003). A total of 483 patient outcomes (353 and 130 patients with acute and acute-on-chronic liver failure, respectively) from 12 randomized trials were assessed. It was concluded that these systems had no effect on mortality in patients with acute liver failure, but a 33% reduction in mortality was seen in patients with acute-on-chronic liver failure. A challenge in the development of a liver support device is the reality that good trials are difficult to design and execute.

The variability in devices and cells, setup of the treatments, patients, and in the outcome parameters used makes it difficult to compare the clinically applied liver support systems. The small number of clinical studies performed thus far are not sufficient to draw definitive conclusions with respect to improvements in the therapy of patients with acute or acute-on-chronic liver failure. Clear aetiology of liver disease should also be considered in the choice of the liver support systems available. Therefore, more complete characterization of the safety and efficacy of the BAL systems requires completion of the Phase I/II safety/efficacy evaluation.

Future perspectives

Multiple hepatocyte culture models and bioreactor constructions are currently available for the development of liver support systems. Although their results collected in many experimental and clinical trials are encouraging, the field is still in its initial stages. Their future use will depend on the choice and stabilization of the cellular component. Thus, a better understanding of hepatocyte–matrix interactions, flow and mass transport across membranes and biomaterials, and host response is still required and will probably result in the development of a new generation of liver assist devices. In addition, immunological problems with the use of xenogeneic hepatocytes and some characteristic differences between human and

animal hepatocytes may necessitate the use of allogeneic materials. Their limited availability has led to the potential application of hepatic stem cells in liver support systems and in liver regeneration. Stem cell research is currently one of the most important fields and its application in tissue engineering bodes well for the future.

Alternatively, the development of bioartificial tissues has also made progress in a number of fields. We have focused on the development of bioreactors in the field of cardiovascular, bone, cartilage, and skin. Such bioreactors are designed for the use of autologous cells and for generating an implant. In contrast, a liver bioreactor is a hybrid device aimed at bridging to transplantation or ideally to autologous liver regeneration. The limited access to autologous liver tissue and the preferred scenario of autologous liver regeneration without transplantation substantiate this concept further. We use primary liver cells and focus on identifying the mechanisms of liver regeneration *in vivo* with the aim of replicating these *in vitro*. Once such mechanisms are elucidated and used for therapeutic reasons *in vitro* or *in vivo*, the next generation of liver support systems will enter the stage of preclinical and clinical testing and thereby overcome the current stagnation in the field.

References

Auth M.K., Okamoto M., Ishida Y., Keogh A., Auth S.H., Gerlach J., Encke A., McMaster P. and Strain A.J. 1998. Maintained function of primary human hepatocytes by cellular interactions in coculture: implications for liver support systems. Transpl. Int. 11(Suppl. 1): S439–S443.

Babensee J.E., De Boni U. and Sefton M.V. 1992. Morphological assessment of hepatoma cells (HepG2) microencapsulated in a HEMA-MMA copolymer with and without Matrigel. J. Biomed. Mater. Res. 26: 1401–1418.

Bader A., Fruhauf N., Zech K., Haverich A. and Borlak J.T. 1998. Development of a small-scale bioreactor for drug metabolism studies maintaining hepatospecific functions. Xenobiotica 28: 815–825.

Bader A., Knop E., Kern A., Boker K., Fruhauf N., Crome O., Esselmann H., Pape C., Kempka G. and Sewing K.F. 1996. 3-D coculture of hepatic sinusoidal cells with primary hepatocytes-design of an organotypical model. Exp. Cell Res. 226: 223–233.

Bader A., Rinkes I.H., Closs E.I., Ryan C.M., Toner M., Cunningham J.M., Tompkins R.G. and Yarmush M.L. 1992. A stable long-term hepatocyte culture system for studies of physiologic processes: cytokine stimulation of the acute phase response in rat and human hepatocytes. Biotechnol. Prog. 8: 219–225.

Baquerizo A., Mhoyan A., Kearns-Jonker M., Arnaout W.S., Shackleton C., Busuttil R.W., Demetriou A.A. and Cramer D.V. 1999. Characterization of human xenoreactive antibodies in liver failure patients exposed to pig hepatocytes after bioartificial liver treatment: an ex vivo model of pig to human xenotransplantation. Transplantation 67: 5–18.

Ben-Ze'ev A., Robinson G.S., Bucher N.L. and Farmer S.R. 1988. Cell–cell and cell–matrix interactions differentially regulate the expression of hepatic and cytoskeletal genes in primary cultures of rat hepatocytes. Proc. Natl. Acad. Sci. U.S.A. 85: 2161–2165.

Black D., Lyman S., Heider T.R. and Behrns K.E. 2004. Molecular and cellular features of hepatic regeneration. J. Surg. Res. 117: 306–315.

Blusch J.H., Patience C. and Martin U. 2002. Pig endogenous retroviruses and xenotransplantation. Xenotransplantation 9: 242–251.

Braet F., Shleper M., Paizi M., Brodsky S., Kopeiko N., Resnick N. and Spira G. 2004. Liver sinusoidal endothelial cell modulation upon resection and shear stress in vitro. Comp. Hepatol. 3: 7.

Bucher N.L., Robinson G.S. and Farmer S.R. 1990. Effects of extracellular matrix on hepatocyte growth and gene expression: implications for hepatic regeneration and the repair of liver injury. Semin. Liver Dis. 10: 11–19.

Canaple L., Nurdin N., Angelova N., Hunkeler D. and Desvergne B. 2001. Development of a coculture model of encapsulated cells. Ann. NY Acad. Sci. 944: 350–361.

Cascio S.M. 2001. Novel strategies for immortalization of human hepatocytes. Artif. Organs 25: 529–538.

Costa R.H., Kalinichenko V.V., Holterman A.X. and Wang X. 2003. Transcription factors in liver development, differentiation, and regeneration. Hepatology 38: 1331–1347.

Curcio E., De Bartolo L., Barbieri G., Rende M., Giorno L., Morelli S. and Drioli E. 2005. Diffusive and convective transport through hollow fiber membranes for liver cell culture. J. Biotechnol. 117: 309–321.

David B., Dore E., Jaffrin M.Y. and Legallais C. 2004. Mass transfers in a fluidized bed bioreactor using alginate beads for a future bioartificial liver. Int. J. Artif. Organs 27: 284–293.

De Bartolo L. and Bader A. 2001. Review of a flat membrane bioreactor as a bioartificial liver. Ann. Transplant. 6: 40–46.

De Bartolo L., Jarosch-Von Schweder G., Haverich A. and Bader A. 2000. A novel full-scale flat membrane bioreactor utilizing porcine hepatocytes: cell viability and tissue-specific functions. Biotechnol. Prog. 16: 102–108.

De Bartolo L., Morelli S., Bader A. and Drioli E. 2002. Evaluation of cell behaviour related to physico-chemical properties of polymeric membranes to be used in bioartificial organs. Biomaterials 23: 2485–2497.

De Bartolo L., Morelli S., Lopez L.C., Giorno L., Campana C., Salerno S., Rende M., Favia P., Detomaso L., Gristina R., d'Agostino R. and Drioli E. 2005. Biotransformation and liver-specific functions of human hepatocytes in culture on RGD-immobilized plasma-processed membranes. Biomaterials 26: 4432–4441.

De Bartolo L., Morelli S., Rende M., Gordano A. and Drioli E. 2004. New modified polyetheretherketone membrane for liver cell culture in biohybrid systems: adhesion and specific functions of isolated hepatocytes. Biomaterials 25: 3621–3629.

De Leeuw A.M., Brouwer A. and Knook D.L. 1990. Sinusoidal endothelial cells of the liver: fine structure and function in relation to age. J. Electron. Microsc. Tech. 14: 218–236.

De Vos P., De Haan B. and Van Schilfgaarde R. 1997. Effect of the alginate composition on the biocompatibility of alginate-polylysine microcapsules. Biomaterials 18: 273–278.

Demetriou A.A., Brown R.S., Busuttil R.W., Fair J., McGuire B.M., Rosenthal P., Am Esch J.S., Lerut J., Nyberg S.L., Salizzoni M., Fagan E.A., de Hemptinne B., Broelsch C.E., Muraca M., Salmeron J.M., Rabkin J.M., Metselaar H.J., Pratt D., La Mata M., McChesney L.P., Everson G.T., Lavin P.T., Stevens A.C., Pitkin Z. and Solomon B.A. 2004. Prospective, randomized, multicenter, controlled trial of a bioartificial liver in treating acute liver failure. Ann. Surg. 239: 660–667.

Dixit V., Darvasi R., Arthur M., Lewin K. and Gitnick G. 1993. Cryopreserved microencapsulated hepatocytes–transplantation studies in Gunn rats. Transplantation 55: 616–622.

Dixit V. and Gitnick G. 1998. The bioartificial liver: state-of-the-art. Eur. J. Surg. 582(Suppl.): S71–S76.

Dore E. and Legallais C. 1999. A new concept of bioartificial liver based on a fluidized bed bioreactor. Ther. Apher. 3: 264–267.

Dou M., de Sousa G., Lacarelle B., Placidi M., la Porte P., Domingo M., Lafont H. and Rahmani R. 1992. Thawed human hepatocytes in primary culture. Cryobiology 29: 454–469.

Dunn J.C., Tompkins R.G. and Yarmush M.L. 1991. Long-term in vitro function of adult hepatocytes in a collagen sandwich configuration. Biotechnol. Prog. 7: 237–245.

Dunn J.C., Tompkins R.G. and Yarmush M.L. 1992. Hepatocytes in collagen sandwich: evidence for transcriptional and translational regulation. J. Cell Biol. 116: 1043–1053.

Dunn J.C., Yarmush M.L., Koebe H.G. and Tompkins R.G. 1989. Hepatocyte function and extracellular matrix geometry: long-term culture in a sandwich configuration. Faseb. J. 3: 174–177.

Ellis A.J., Hughes R.D., Wendon J.A., Dunne J., Langley P.G., Kelly J.H., Gislason G.T., Sussman N.L. and Williams R. 1996. Pilot-controlled trial of the extracorporeal liver assist device in acute liver failure. Hepatology 24: 1446–1451.

Elvevold K.H., Nedredal G.I., Revhaug A. and Smedsrod B. 2004. Scavenger properties of cultivated pig liver endothelial cells. Comp. Hepatol. 3: 4.

Enomoto K., Nishikawa Y., Omori Y., Tokairin T., Yoshida M., Ohi N., Nishimura T., Yamamoto Y. and Li Q. 2004. Cell biology and pathology of liver sinusoidal endothelial cells. Med. Electron. Microsc. 37: 208–215.

Eschbach E., Chatterjee S.S., Noldner M., Gottwald E., Dertinger H., Weibezahn K.F. and Knedlitschek G. 2005. Microstructured scaffolds for liver tissue cultures of high cell density: morphological and biochemical characterization of tissue aggregates. J. Cell Biochem. 95: 243–255.

Evenepoel P., Laleman W., Wilmer A., Claes K., Maes B., Kuypers D., Bammens B., Nevens F. and Vanrenterghem Y. 2005. Detoxifying capacity and kinetics of prometheus – a new extracorporeal system for the treatment of liver failure. Blood Purif. 23: 349–358.

Fishman J.A. and Patience C. 2004. Xenotransplantation: infectious risk revisited. Am. J. Transplant. 4: 1383–1390.

Flendrig L.M., Calise F., Di Florio E., Mancini A., Ceriello A., Santaniello W., Mezza E., Sicoli F., Belleza G., Bracco A., Cozzolino S., Scala D., Mazzone M., Fattore M., Gonzales E. and Chamuleau R.A. 1999. Significantly improved survival time in pigs with complete liver ischemia treated with a novel bioartificial liver. Int. J. Artif. Organs 22: 701–709.

Flendrig L.M., la Soe J.W., Jorning G.G., Steenbeek A., Karlsen O.T., Bovee W.M., Ladiges N.C., te Velde A.A. and Chamuleau R.A. 1997. 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. 26: 1379–1392.

Fremond B., Malandain C., Guyomard C., Chesne C., Guillouzo A. and Campion J.P. 1993. Correction of bilirubin conjugation in the Gunn rat using hepatocytes immobilized in alginate gel beads as an extracorporeal bioartificial liver. Cell Transplant. 2: 453–460.

Fruhauf N.R., Oldhafer K.J., Holtje M., Kaiser G.M., Fruhauf J.H., Stavrou G.A., Bader A. and Broelsch C.E. 2004. A bioartificial liver support system using primary hepatocytes: a preclinical study in a new porcine hepatectomy model. Surgery 136: 47–56.

Gan J.H., Zhou X.Q., Qin A.L., Luo E.P., Zhao W.F., Yu H. and Xu J. 2005. Hybrid artificial liver support system for treatment of severe liver failure. World J. Gastroenterol. 11: 890–894.

Gerlach J.C. 1996. Development of a hybrid liver support system: a review. Int. J. Artif. Organs 19: 645–654.

Gerlach J.C., Kloppel K., Muller C., Schnoy N., Smith M.D. and Neuhaus P. 1993. Hepatocyte aggregate culture technique for bioreactors in hybrid liver support systems. Int. J. Artif. Organs 16: 843–846.

Gerlach J.C., Mutig K., Sauer I.M., Schrade P., Efimova E., Mieder T., Naumann G., Grunwald A., Pless G., Mas A., Bachmann S., Neuhaus P. and Zeilinger K. 2003. Use of primary human liver cells originating from discarded grafts in a bioreactor for liver support therapy and the prospects of culturing adult liver stem cells in bioreactors: a morphologic study. Transplantation 76: 781–786.

Gimson A.E. 1996. Fulminant and late onset hepatic failure. Br. J. Anaesth. 77: 90–98.

Glicklis R., Merchuk J.C. and Cohen S. 2004. Modeling mass transfer in hepatocyte spheroids via cell viability, spheroid size, and hepatocellular functions. Biotechnol. Bioeng. 86: 672–680.

Glicklis R., Shapiro L., Agbaria R., Merchuk J.C. and Cohen S. 2000. Hepatocyte behavior within three-dimensional porous alginate scaffolds. Biotechnol. Bioeng. 67: 344–353.

Gregory P.G., Connolly C.K., Toner M. and Sullivan S.J. 2000. In vitro characterization of porcine hepatocyte function. Cell Transplant. 9: 1–10.

Guillouzo A., Rialland L., Fautrel A. and Guyomard C. 1999. Survival and function of isolated hepatocytes after cryopreservation. Chem. Biol. Interact. 121: 7–16.

Hasegawa H., Shimada M., Gion T., Ijima H., Nakazawa K., Funatsu K. and Sugimachi K. 1999. Modulation of immunologic reactions between cultured porcine hepatocytes and human sera. ASAIO J. 45: 392–396.

He Z.P., Tan W.Q., Tang Y.F. and Feng M.F. 2003. Differentiation of putative hepatic stem cells derived from adult rats into mature hepatocytes in the presence of epidermal growth factor and hepatocyte growth factor. Differentiation 71: 281–290.

Hengstler J.G., Ringel M., Biefang K., Hammel S., Milbert U., Gerl M., Klebach M., Diener B., Platt K.L., Bottger T., Steinberg P. and Oesch F. 2000. Cultures with cryopreserved hepatocytes: applicability for studies of enzyme induction. Chem. Biol. Interact. 125: 51–73.

Hoekstra R. and Chamuleau R.A. 2002. Recent developments on human cell lines for the bioartificial liver. Int. J. Artif. Organs 25: 182–191.

Honiger J., Sarkis R., Baudrimont M., Delelo R., Chafai N., Benoist S., Sarkis K., Balladur P., Capeau J. and Nordlinger B. 2000. Semiautomatic macroencapsulation of large numbers of porcine hepatocytes by coextrusion with a solution of AN69 polymer. Biomaterials 21: 1269–1274.

Hughes R.D., Nicolaou N., Langley P.G., Ellis A.J., Wendon J.A. and Williams R. 1998. Plasma cytokine levels and coagulation and complement activation during use of the extracorporeal liver assist device in acute liver failure. Artif. Organs 22: 854–858.

Irgang M., Sauer I.M., Karlas A., Zeilinger K., Gerlach J.C., Kurth R., Neuhaus P. and Denner J. 2003. Porcine endogenous retroviruses: no infection in patients treated with a bioreactor based on porcine liver cells. J. Clin. Virol. 28: 141–154.

Isom H.C., Secott T., Georgoff I., Woodworth C. and Mummaw J. 1985. Maintenance of differentiated rat hepatocytes in primary culture. Proc. Natl. Acad. Sci. U.S.A. 82: 3252–3256.

Jasmund I., Langsch A., Simmoteit R. and Bader A. 2002. Cultivation of primary porcine hepatocytes in an OXY-HFB for use as a bioartificial liver device. Biotechnol. Prog. 18: 839–846.

Jauregui H.O., Mullon C.J., Trenkler D., Naik S., Santangini H., Press P., Muller T.E. and Solomon B.A. 1995. In vivo evaluation of a hollow fiber liver assist device. Hepatology 21: 460–469.

Jauregui H.O., Naik S., Santangini H., Pan J., Trenkler D. and Mullon C. 1994. Primary cultures of rat hepatocytes in hollow fiber chambers. In Vitro Cell Dev Biol. Anim. 30: 23–29.

Joly A., Desjardins J.F., Fremond B., Desille M., Campion J.P., Malledant Y., Lebreton Y., Semana G., Edwards-Levy F., Levy M.C. and Clement B. 1997. Survival, proliferation, and functions of porcine hepatocytes encapsulated in coated alginate beads: a step toward a reliable bioartificial liver. Transplantation 63: 795–803.

Kang Y.H., Berthiaume F., Nath B.D. and Yarmush M.L. 2004. Growth factors and nonparenchymal cell conditioned media induce mitogenic responses in stable long-term adult rat hepatocyte cultures. Exp. Cell Res. 293: 239–247.

King A., Strand B., Rokstad A.M., Kulseng B., Andersson A., Skjak-Braek G. and Sandler S. 2003. Improvement of the biocompatibility of alginate/poly-L-lysine/alginate microcapsules by the use of epimerized alginate as a coating. J. Biomed. Mater. Res. (Part) A. 64: 533–539.

Kjaergard L.L., Liu J., Als-Nielsen B. and Gluud C. 2003. Artificial and bioartificial support systems for acute and acute-on-chronic liver failure: a systematic review. JAMA 289: 217–222.

Kmieć Z. 2001. Cooperation of liver cells in health and disease. In: Beck F., Christ B., Kriz W., Kummer W., Marani E., Putz R., Sano Y., Schiebler T.H., Schoenwolf G.C. and Zilles K. (eds), Advances in Anatomy Embryology and Cell Biology, Vol. 161. Springer-Verlag, Berlin, Heidelberg, New York, pp. 1–149.

Kobayashi N., Okitsu T., Nakaji S. and Tanaka N. 2003. Hybrid bioartificial liver: establishing a reversibly immortalized human hepatocyte line and developing a bioartificial liver for practical use. J. Artif. Organs 6: 236–244.

Koide N., Sakaguchi K., Koide Y., Asano K., Kawaguchi M., Matsushima H., Takenami T., Shinji T., Mori M. and Tsuji T. 1990. Formation of multicellular spheroids composed of adult rat hepatocytes in dishes with positively charged surfaces and under other nonadherent environments. Exp. Cell Res. 186: 227–235.

Koniaris L.G., McKillop I.H., Schwartz S.I. and Zimmers T.A. 2003. Liver regeneration. J. Am. Coll. Surg. 197: 634–659.

Krisper P., Haditsch B., Stauber R., Jung A., Stadlbauer V., Trauner M., Holzer H. and Schneditz D. 2005. In vivo quantification of liver dialysis: comparison of albumin dialysis and fractionated plasma separation. J. Hepatol. 43: 451–457.

Kuddus R., Patzer J.F., Lopez R., Mazariegos G.V., Meighen B., Kramer D.J. and Rao A.S. 2002. Clinical and laboratory evaluation of the safety of a bioartificial liver assist device for potential transmission of porcine endogenous retrovirus. Transplantation 73: 420–429.

Lacik I., Brissova M., Anilkumar A.V., Powers A.C. and Wang T. 1998. New capsule with tailored properties for the encapsulation of living cells. J. Biomed. Mater. Res. 39: 52–60.

Langsch A. and Bader A. 2001. Longterm stability of phase I and phase II enzymes of porcine liver cells in flat membrane bioreactors. Biotechnol. Bioeng. 76: 115–125.

Lee K.W., Park J.B., Yoon J.J., Lee J.H., Kim S.Y., Jung H.J., Lee S.K., Kim S.J., Lee H.H., Lee D.S. and Joh J.W. 2004. The viability and function of cryopreserved hepatocyte spheroids with different cryopreservation solutions. Transplant. Proc. 36: 2462–2463.

Lee W.M. 1993. Acute liver failure. N. Engl. J. Med. 329: 1862–1872.

Legallais C., Dore E. and Paullier P. 2000. Design of a fluidized bed bioartificial liver. Artif. Organs 24: 519–525.

Lorenti A., Barbich M., de Santibanes M., Ielpi M., Vazquez J.C., Sorroche P. and Argibay P. 2003. Ammonium detoxification performed by porcine hepatocyte spheroids in a bioartificial liver for pediatric use: preliminary report. Artif. Organs 27: 665–670.

Louha M., Poussin K., Ganne N., Zylberberg H., Nalpas B., Nicolet J., Capron F., Soubrane O., Vons C., Pol S., Beaugrand M, Berthelot P., Franco D., Trinchet J.C., Brechot C. and Paterlini P. 1997. Spontaneous and iatrogenic spreading of liver-derived cells into peripheral blood of patients with primary liver cancer. Hepatology 26: 998–1005.

Lowes K.N., Croager E.J., Olynyk J.K., Abraham L.J. and Yeoh G.C. 2003. Oval cell-mediated liver regeneration: Role of cytokines and growth factors. J. Gastroenterol. Hepatol. 18: 4–12.

Mai G., Huy N.T., Morel P., Mei J., Bosco D., Berney T., Majno P., Mentha G., Trono D. and Buhler L.H. 2005. Treatment of fulminant liver failure by transplantation of microencapsulated primary or immortalized xenogeneic hepatocytes. Transplant. Proc. 37: 527–529.

Martinez-Hernandez A. and Amenta P.S. 1993. The hepatic extracellular matrix. I. Components and distribution in

normal liver. Virchows Arch. A. Pathol. Anat. Histopathol. 423: 1–11.

Matsushita T., Yagi T., Hardin J.A., Cragun J.D., Crow F.W., Bergen H.R., Gores G.J. and Nyberg S.L. 2003. Apoptotic cell death and function of cryopreserved porcine hepatocytes in a bioartificial liver. Cell Transplant. 12: 109–121.

Mazariegos G.V., Kramer D.J., Lopez R.C., Shakil A.O., Rosenbloom A.J., DeVera M., Giraldo M., Grogan T.A., Zhu Y., Fulmer M.L., Amiot B.P. and Patzer J.F. 2001. Safety observations in phase I clinical evaluation of the Excorp Medical Bioartificial Liver Support System after the first four patients. ASAIO J. 47: 471–475.

McClelland R.E., MacDonald J.M. and Coger R.N. 2003. Modeling O_2 transport within engineered hepatic devices. Biotechnol. Bioeng. 82: 12–27.

McLaughlin B.E., Tosone C.M., Custer L.M. and Mullon C. 1999. Overview of extracorporeal liver support systems and clinical results. Ann. NY Acad. Sci. 875: 310–325.

Michalopoulos G.K., Bowen W.C., Mule K. and Stolz D.B. 2001. Histological organization in hepatocyte organoid cultures. Am. J. Pathol. 159: 1877–1887.

Michalopoulos G.K. and DeFrances M.C. 1997. Liver regeneration. Science 276: 60–66.

Mitzner S.R., Stange J., Klammt S., Peszynski P. and Schmidt R. 2001. Albumin dialysis using the molecular adsorbent recirculating system. Curr. Opin. Nephrol. Hypertens. 10: 777–783.

Miura Y., Akimoto T. and Yagi K. 1988. Liver functions in hepatocytes entrapped within calcium alginate. Ann. NY Acad. Sci. 542: 521–532.

Morsiani E., Brogli M., Galavotti D., Bellini T., Ricci D., Pazzi P. and Puviani A.C. 2001. Long-term expression of highly differentiated functions by isolated porcine hepatocytes perfused in a radial–flow bioreactor. Artif. Organs 25: 740–748.

Morsiani E., Brogli M., Galavotti D., Pazzi P., Puviani A.C. and Azzena G.F. 2002a. Biologic liver support: optimal cell source and mass. Int. J. Artif. Organs 25: 985–993.

Morsiani E., Pazzi P., Puviani A.C., Brogli M., Valieri L., Gorini P., Scoletta P., Marangoni E., Ragazzi R., Azzena G., Frazzoli E., Di Luca D. and Cassai E. 2002b. Early experiences with a porcine hepatocyte-based bioartificial liver in acute hepatic failure patients. Int. J. Artif. Organs 25: 192–202.

Muraca M., Vilei M.T., Zanusso E., Ferraresso C., Granato A., Doninsegna S., Dal Monte R., Carraro P. and Carturan G. 2000. Encapsulation of hepatocytes by SiO(2). Transplant Proc. 32: 2713–2714.

Muto Y., Nouri-Aria K.T., Meager A., Alexander G.J., Eddleston A.L. and Williams R. 1988. Enhanced tumour necrosis factor and interleukin-1 in fulminant hepatic failure. Lancet 2: 72–74.

Nagy A. 2000. Cre recombinase: the universal reagent for genome tailoring. Genesis 26: 99–109.

Nyberg S.L., Hibbs J.R., Hardin J.A., Germer J.J. and Persing D.H. 1999. Transfer of porcine endogenous retrovirus across hollow fiber membranes: significance to a bioartificial liver. Transplantation 67: 1251–1255.

Nyberg S.L., Mann H.J., Hu M.Y., Payne W.D., Hu W.S., Cerra F.B. and Remmel R.P. 1996. Extrahepatic metabolism of 4-methylumbelliferone and lidocaine in the anhepatic rabbit. Drug Metab. Dispos. 24: 643–648.

Nyberg S.L., Platt J.L., Shirabe K., Payne W.D., Hu W.S. and Cerra F.B. 1992a. Immunoprotection of xenocytes in a hollow fiber bioartificial liver. ASAIO J. 38: M463–M467.

Nyberg S.L., Shatford R.A., Hu W.S., Payne W.D. and Cerra F.B. 1992b. Hepatocyte culture systems for artificial liver support: implications for critical care medicine (bioartificial liver support). Crit. Care Med. 20: 1157–1168.

Nyberg S.L., Shatford R.A., Payne W.D., Hu W.S. and Cerra F.B. 1992c. Primary culture of rat hepatocytes entrapped in cylindrical collagen gels: an in vitro system with application to the bioartificial liver. Rat hepatocytes cultured in cylindrical collagen gels. Cytotechnology 10: 205–215.

Nyberg S.L., Shirabe K., Peshwa M.V., Sielaff T.D., Crotty P.L., Mann H.J., Remmel R.P., Payne W.D., Hu W.S. and Cerra F.B. 1993. Extracorporeal application of a gel-entrapment, bioartificial liver: demonstration of drug metabolism and other biochemical functions. Cell Transplant. 2: 441–452.

Orive G., Hernandez R.M., Gascon A.R., Calafiore R., Chang T.M., De Vos P., Hortelano G., Hunkeler D., Lacik I., Shapiro A.M. and Pedraz J.L. 2003. Cell encapsulation: promise and progress. Nat. Med. 9: 104–107.

Orive G., Hernandez R.M., Rodriguez Gascon A., Calafiore R., Chang T.M., de Vos P., Hortelano G., Hunkeler D., Lacik I. and Pedraz J.L 2004. History, challenges and perspectives of cell microencapsulation. Trends Biotechnol. 22: 87–92.

Pahernik S.A., Thasler W.E., Doser M., Gomez-Lechon M.J., Castell M.J., Planck H. and Koebe H.G. 2001. High density culturing of porcine hepatocytes immobilized on nonwoven polyurethane-based biomatrices. Cells Tissues Organs 168: 170–177.

Pitkin Z. and Mullon C. 1999. Evidence of absence of porcine endogenous retrovirus (PERV) infection in patients treated with a bioartificial liver support system. Artif. Organs 23: 829–833.

Quek C.H., Li J., Sun T., Chan M.L., Mao H.Q., Gan L.M., Leong K.W. and Yu H. 2004. Photo-crosslinkable microcapsules formed by polyelectrolyte copolymer and modified collagen for rat hepatocyte encapsulation. Biomaterials 25: 3531–3540.

Rahman T. and Hodgson H. 2001. Clinical management of acute hepatic failure. Intensive Care Med. 27: 467–476.

Ramadori G. and Armbrust T. 2001. Cytokines in the liver. Eur. J. Gastroenterol. Hepatol. 13: 777–784.

Rifai K., Bahr M., Schneider A., Ott M. and Mann M. 2003. Neue Verfahren in der Leberersatztherapie. Medizinische Klinik 98: 750–753.

Runge D., Runge D.M., Jager D., Lubecki K.A., Beer Stolz D., Karathanasis S., Kietzmann T., Strom S.C., Jungermann K., Fleig W.E. and Michalopoulos G.K. 2000. Serum-free, long-term cultures of human hepatocytes: maintenance of cell morphology, transcription factors, and liver-specific functions. Biochem. Biophys. Res. Commun. 269: 46–53.

Saad B., Scholl F.A., Thomas H., Schawalder H., Streit V., Waechter F. and Maier P. 1993. Crude liver membrane fractions and extracellular matrix components as substrata regulate differentially the preservation and inducibility of cytochrome P-450 isoenzymes in cultured rat hepatocytes. Eur. J. Biochem. 213: 805–814.

Sakai Y., Naruse K., Nagashima I., Muto T. and Suzuki M. 1996. Large-scale preparation and function of porcine hepatocyte spheroids. Int. J. Artif. Organs 19: 294–301.

Sato Y., Tsukada K. and Hatakeyama K. 1999. Role of shear stress and immune responses in liver regeneration after a partial hepatectomy. Surg. Today 29: 1–9.

Sauer I.M. and Gerlach J.C. 2002. Modular extracorporeal liver support. Artif. Organs 26: 703–706.

Sauer I.M., Kardassis D., Zeillinger K., Pascher A., Gruenwald A., Pless G., Irgang M., Kraemer M., Puhl G., Frank J., Muller A.R., Steinmuller T., Denner J., Neuhaus P. and Gerlach J.C. 2003. Clinical extracorporeal hybrid liver support–phase I study with primary porcine liver cells. Xenotransplantation 10: 460–469.

Sauer I.M., Obermeyer N., Kardassis D., Theruvath T. and Gerlach J.C. 2001. Development of a hybrid liver support system. Ann. NY Acad. Sci. 944: 308–319.

Sauer I.M., Zeilinger K., Obermayer N., Pless G., Grunwald A., Pascher A., Mieder T., Roth S., Goetz M., Kardassis D., Mas A., Neuhaus P. and Gerlach J.C. 2002. Primary human liver cells as source for modular extracorporeal liver support–a preliminary report. Int. J. Artif. Organs 25: 1001–1005.

Schoen J.M., Wang H.H., Minuk G.Y. and Lautt W.W. 2001. Shear stress-induced nitric oxide release triggers the liver regeneration cascade. Nitric Oxide 5: 453–464.

Schuetz E.G., Li D., Omiecinski C.J., Muller-Eberhard U., Kleinman H.K., Elswick B. and Guzelian P.S. 1988. Regulation of gene expression in adult rat hepatocytes cultured on a basement membrane matrix. J. Cell Physiol. 134: 309–323.

Semino C.E., Merok J.R., Crane G.G., Panagiotakos G. and Zhang S. 2003. Functional differentiation of hepatocyte-like spheroid structures from putative liver progenitor cells in three-dimensional peptide scaffolds. Differentiation 71: 262–270.

Seo S.J., Akaike T., Choi Y.J., Shirakawa M., Kang I.K. and Cho C.S. 2005. Alginate microcapsules prepared with xyloglucan as a synthetic extracellular matrix for hepatocyte attachment. Biomaterials 26: 3607–3615.

Serandour A.L., Loyer P., Garnier D., Courselaud B., Theret N., Glaise D., Guguen-Guillouzo C. and Corlu A. 2005. TNFα-mediated extracellular matrix remodeling is required for multiple division cycles in rat hepatocytes. Hepatology 41: 478–486.

Shito M., Tilles A.W., Tompkins R.G., Yarmush M.L. and Toner M. 2003. Efficacy of an extracorporeal flat-plate bioartificial liver in treating fulminant hepatic failure. J. Surg. Res. 111: 53–62.

Sielaff T.D., Hu M.Y., Rao S., Groehler K., Olson D., Mann H.J., Remmel R.P., Shatford R.A., Amiot B. and Hu W.S. et al. 1995. A technique for porcine hepatocyte harvest and description of differentiated metabolic functions in static culture. Transplantation 59: 1459–1463.

Stange J. and Mitzner S. 1996. Hepatocyte encapsulation–initial intentions and new aspects for its use in bioartificial liver support. Int. J. Artif. Organs 19: 45–48.

Sussman N.L., Chong M.G., Koussayer T., He D.E., Shang T.A., Whisennand H.H. and Kelly J.H. 1992. Reversal of fulminant hepatic failure using an extracorporeal liver assist device. Hepatology 16: 60–65.

Sussman N.L. and Kelly J.H. 1993. Improved liver function following treatment with an extracorporeal liver assist device. Artif. Organs 17: 27–30.

Suzuki A., Iwama A., Miyashita H., Nakauchi H. and Taniguchi H. 2003. Role for growth factors and extracellular matrix in controlling differentiation of prospectively isolated hepatic stem cells. Development 130: 2513–2524.

Taub R. 2004. Liver regeneration: from myth to mechanism. Nat. Rev. Mol. Cell Biol. 5: 836–847.

te Velde A.A., Ladiges N.C., Flendrig L.M. and Chamuleau R.A. 1995. Functional activity of isolated pig hepatocytes attached to different extracellular matrix substrates. Implication for application of pig hepatocytes in a bioartificial liver. J. Hepatol. 23: 184–192.

Tong J.Z., Sarrazin S., Cassio D., Gauthier F. and Alvarez F. 1994. Application of spheroid culture to human hepatocytes and maintenance of their differentiation. Biol. Cell 81: 77–81.

Tsiaoussis J., Newsome P.N., Nelson L.J., Hayes P.C. and Plevris J.N. 2001. Which hepatocyte will it be? Hepatocyte choice for bioartificial liver support systems. Liver Transpl. 7: 2–10.

Uludag H., De Vos P. and Tresco P.A. 2000. Technology of mammalian cell encapsulation. Adv. Drug Deliv. Rev. 42: 29–64.

van de Kerkhove M.P., Germans M.R., Deurholt T., Hoekstra R., Joziasse D.H., van Wijk A.C., van Gulik T.M., Chamuleau R.A. and Roos A. 2005a. Evidence for Galα (1–3)Gal expression on primary porcine hepatocytes: implications for bioartificial liver systems. J. Hepatol. 42: 541–547.

van de Kerkhove M.P., Hoekstra R., Chamuleau R.A. and van Gulik T.M. 2004. Clinical application of bioartificial liver support systems. Ann. Surg. 240: 216–230.

van de Kerkhove M.P., Poyck P.P., Deurholt T., Hoekstra R., Chamuleau R.A. and van Gulik T.M. 2005b. Liver support therapy: an overview of the AMC-bioartificial liver research. Dig. Surg. 22: 254–264.

Wake K. 1999. Cell-cell organization and functions of 'sinusoids' in liver microcirculation system. J. Electron. Microsc. (Tokyo) 48: 89–98.

Werner A., Duvar S., Muthing J., Buntemeyer H., Kahmann U., Lunsdorf H. and Lehmann J. 1999. Cultivation and characterization of a new immortalized human hepatocyte cell line, HepZ, for use in an artificial liver support system. Ann. NY Acad. Sci. 875: 364–368.

Wilkening S., Stahl F. and Bader A. 2003. Comparison of primary human hepatocytes and hepatoma cell line HepG2 with regard to their biotransformation properties. Drug Metab. Dispos. 31: 1035–1042.

Xu J., Ma M. and Purcell W.M. 2003. Biochemical and functional changes of rat liver spheroids during spheroid formation and maintenance in culture: II. nitric oxide synthesis and related changes. J. Cell Biochem. 90: 1176–1185.

Yamada K., Kamihira M. and Iijima S. 2001. Self-organization of liver constitutive cells mediated by artificial matrix and improvement of liver functions in long-term culture. Biochem. Eng. J. 8: 135–143.

Yamashita Y., Shimada M., Tsujita E., Shirabe K., Ijima H., Nakazawa K., Sakiyama R., Fukuda J., Funatsu K. and Sugimachi K. 2003. Efficacy of a larger version of the hybrid artificial liver support system using a polyurethane foam/spheroid packed-bed module in a warm ischemic liver failure pig model for preclinical experiments. Cell Transplant. 12: 101–107.