Adv Biochem Engin/Biotechnol (2009) 115: 117–143
DOI: 10.1007/10_2008_34

Published online: 28 May 2009

Transport Advances in Disposable Bioreactors for Liver Tissue Engineering

Gerardo Catapano, John F. Patzer II, and Jörg Christian Gerlach

Abstract Acute liver failure (ALF) is a devastating diagnosis with an overall survival of approximately 60%. Liver transplantation is the therapy of choice for ALF patients but is limited by the scarce availability of donor organs. The prognosis of ALF patients may improve if essential liver functions are restored during liver failure by means of auxiliary methods because liver tissue has the capability to regenerate and heal. Bioartificial liver (BAL) approaches use liver tissue or cells to provide ALF patients with liver-specific metabolism and synthesis products necessary to relieve some of the symptoms and to promote liver tissue regeneration. The most promising BAL treatments are based on the culture of tissue engineered (TE) liver constructs, with mature liver cells or cells that may differentiate into hepatocytes to perform liver-specific functions, in disposable continuous-flow bioreactors. In fact, adult hepatocytes perform all essential liver functions. Clinical evaluations of the proposed BALs show that they are safe but have not clearly proven the efficacy of treatment as compared to standard supportive treatments. Ambiguous clinical results, the time loss of cellular activity during treatment, and the presence of a necrotic core in the cell compartment of many bioreactors suggest that improvement of transport of nutrients, and metabolic wastes and products to or from the cells in the bioreactor is critical for the development of therapeutically

G. Catapano (✉)
Department of Chemical Engineering and Materials, University of Calabria, Rende (CS), Italy
e-mail: catapano@unical.it

J.F. Patzer II
Department of Surgery, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA and Department of Bioengineering, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA

J.C. Gerlach
Department of Surgery, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA; Department of Bioengineering, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA; and Charité, Medical Faculty of the Berlin Universities, Berlin, Germany

effective BALs. In this chapter, advanced strategies that have been proposed over to improve mass transport in the bioreactors at the core of a BAL for the treatment of ALF patients are reviewed.

Keywords Bioartificial, Bioreactor, Cell, Disposable, Liver, Mass, Transport.

Contents

1 Introduction

Acute liver failure (ALF) is a devastating diagnosis. Overall survival associated with best medical care has improved from approximately 20% in the 1970s to approximately 60% during the past decade [1]. Survival is etiology dependent, ranging from ~25% for drug-induced ALF, hepatitis B, and cryptogenic cases to ~60% for acetaminophen overdose, hepatitis A, and ischemia [2]. Liver transplantation (LTx), with 1-year survival rates of 60–80% for ALF patients, is the therapy of choice for ALF patients [3, 4]. Availability of organs for LTx is problematic: 6,530 patients out of 17,298 on the wait list received a liver transplant (38%) in the United States in 2006–2007 (www.ustransplant.org, 2008). Etiologies and transplant rates vary from country to country [5, 6], but reflect similar trends. In many countries, the high social costs of transplantation and the associated year-long immunosuppressive therapy also pose serious ethical questions on the eligibility criteria for liver transplantation and often further limit the number of LTx procedures [7].

The prognosis of many of ALF patients may improve without the need for LTx if essential liver functions are restored during liver failure by means of auxiliary methods [1, 2, 8]. In fact, liver tissue has the capability to regenerate and heal [9]. In the last decade, exploitation of this possibility has led to the development of innovative treatments for ALF that include split-liver transplantation, extracorporeal artificial liver (AL) support (nonbiological detoxification), extracorporeal bioartificial liver (BAL) support (cell-based systems), and in vivo tissue or cell transplantation [10]. Success of split-liver transplantation has been reported to be close to that of LTx, but the procedure is not broadly performed and surgery may be complicated by anatomical variations [11].

Extracorporeal AL approaches are directed toward removal of plasma toxins (e.g., ammonia, mercaptans, free phenols, bile acids, benzodiazepines, etc.) that accumulate in ALF patients [12]. To this purpose, hemodialysis, hemoperfusion or

plasmapheresis cartridges and procedures have been used, as clinically available or modified by using albumin (dissolved in the stripping solution or adsorbed in the pores of asymmetric membranes) to facilitate removal of protein-bound toxins [13–18]. However, AL approaches have not yet demonstrated significant improvement over conventional patient management.

BAL approaches use liver tissue or cells to provide ALF patients with liver-specific metabolism and synthesis products necessary to relieve some of the symptoms (e.g., cerebral oedema and bleeding) to promote liver tissue regeneration and, ideally, to provide the whole set of liver-specific biosynthetic and biotransformation functions that the failing liver cannot provide. BAL treatments based on direct perfusion of xenogeneic whole liver or liver slices, or cross-hemodialysis against xenogeneic livers have been reported to have some beneficial effects, but are impractical in the clinical setting [19]. Such xenogeneic approaches also put the ALF patient at risk of massive immune reaction against the xenogeneic organ or released soluble antigens.

The most promising BAL treatments are based on the use of mature liver cells (e.g., primary or immortalized) or cells that may differentiate into hepatocytes (e.g., adult stem cells or progenitor cells) to perform liver-specific functions [20]. In fact, adult hepatocytes have been shown to perform all essential liver functions [21, 22]. Unfortunately, in vitro isolated primary hepatocytes cultured in suspension lose their morphology, depolarize, dedifferentiate, are not able to perform the natural liver functions, and die within a few hours. Even in in vitro cultures that have been shown to stabilize their functions for a few weeks, isolated hepatocytes do not generally proliferate. Available information on the structure–function relationships of healthy and pathological liver tissue is also incomplete.

The challenge in BAL and in vivo tissue transplantation for treatment of ALF patients is design and development of liver tissue patterned after the native liver microarchitecture so as to foster the same cellular and functional relationships that exist in the healthy natural liver. The formidable technical challenge of engineering liver tissue in vitro is reflected in the multicellular and highly hierarchical architecture of the natural liver tissue, with complex vascularization, and the mass of the biological substitute presumed necessary to restore homeostasis in ALF patients. Hepatectomy studies suggest that a tissue engineered (TE) liver construct for effective treatment of ALF needs to perform metabolic functions equivalent to about 10–30% of the natural liver mass for an estimated 200–500 g mass of metabolically active parenchymal liver cells for an adult human [9, 23].

To date, BAL treatments for ALF that have been clinically evaluated are based on TE liver tissue containing natural or transformed liver cells seeded on two- (2D) or three-dimensional (3D) nonresorbable scaffolds that provide the template for cell adhesion, reorganization, proliferation (transformed liver cells), and differentiation. The BAL-TE liver construct is generally cultured in continuous-flow bioreactors that treat the patient's blood or plasma flowing in an extracorporeal loop. Cell sourcing, development, design, operational features, preclinical and clinical performance of the bioreactors and the BALs proposed over the years have been presented and discussed in many review papers [7, 22, 24–30].

Bioreactors that have been proposed as the core of BAL systems testify to the ingenuity of researchers active in the field. Listed in Table 1, the BAL systems differ

Table 1 Continuous-flow bioreactors used for the clinical treatment of acute liver failure (ALF) patients (adapted from [28])

Bioreactor brand name and type	Membrane type and NMWCO	Auxiliary physical treatment	Perfusate and flow rate	Cell number and type	References
Excorp BLSS® Shell-and-tube HF membrane bioreactor with cells in the shell embedded in collagen outside a bundle of membranes and blood flowing in the membrane lumen	Polysulphone 100 kDa	Blood oxygenator	Blood	$8–20 \times 10^9$ (ca. 100 g) porcine hepatocytes	[31, 32]
Vital Therapies ELAD® Shell-and-tube HF membrane bioreactor with cells adherent on the external membrane surface and blood fed to membrane lumen	Cellulose acetate 70 kDa	Blood oxygenator	Blood 150–300 mL min^{-1}	2×10^{10} (ca. 200 g) cells of human epatoblastoma C3A line	[33]
Arbios Systems HepatAssist® Shell-and-tube HF membrane bioreactor with cryopreserved cell clumps adherent on dextran microbeads in the shell and plasma fed to membrane lumen	Microporous polysulphone maximal pore size 0.2 µm	Activated charcoal adsorbent cartridge	Plasma-recirculated at 400 mL min^{-1}	$4–6 \times 10^9$ (ca. 50 g) cryopreserved porcine hepatocytes	[34]
MELS CellModule Interwven four compartment membrane network bioreactor for 3D cell perfusion at tissue densities with repeating units consisting of two overlaid hydrophilic microporous HF membrane mats and a mat of microporous hydrophobic membranes interposed among them for oxygen supply. Cells cultured outside and among the membranes are perfused by	Microporous hydrophilic polyethersulphone pore size 0.2 µm Hydrophobic asymmetric polymethylpentene for plasma oxygenation maximal pore size 0.1 µm	Dialysis and removal of hydrophobic toxins with a concentrated albumin solution possible	Plasma in single pass at 40–60 mL per minor or Plasma recirculated at 400 mL min^{-1}	$2–8 \times 10^{10}$ (ca. 500 g) porcine or human liver cells	[17, 35, 36]
AMC-BAL Cylindrical packed bioreactor with cell-seeded spiral wound polyester nonwoven fabric with HF membranes for oxygen supply, and plasma axially perfusing the fabric	Hydrophobic symmetric polypropylene for plasma oxygenation maximal pore size 0.1 µm		Plasma	2×10^9 porcine cells	[37]
RAnD BAL Annular packed bioreactor with cell seeded spiral wound polyester nonwoven fabric, and plasma radially perfusing the fabric		Bilirubin adsorption cartridge	Plasma	$2–2.3 \times 10^{10}$ porcine hepatocytes	[38]

in the type and mass of cells used, the geometrical and physical–chemical properties of the scaffolds, the cell seeding technique, the bioreactor design and operation, the fluid treated (blood vs plasma), and the possible use of auxiliary devices for the physical treatment of the processed fluid (e.g., to reduce the toxin load on the liver cells or to replenish oxygen in the fluid entering the bioreactor, etc.). Laboratory tests and trials with animal models of ALF have generally shown that BALs are promising alternatives to LTx in the treatment of ALF. A few of the "first generation" BALs have undergone extensive experimental evaluation and are still being tested in the clinical setting but, to date, none of the proposed BALs has yet been approved for clinical treatment of ALF or chronic liver failure.

Five out of the six BAL systems that have been clinically evaluated use primary porcine hepatocytes (Arbios HepatAssist®, MELS CellModule, Excorp BLSS, AMC-BAL, and RAnD BAL). The Vital Therapies ELAD® uses the tumor-derived C3A cell line, a subclone of the HepG2 cell line. In four out of six bioreactors cells are seeded outside perm-selective hollow fiber membranes (in the extracapillary space of the bioreactor), with whole blood or plasma flowing in the membrane lumen (Arbios HepatAssist®, MELS CellModule, Excorp BLSS, Vital Therapies ELAD®). In two of them (AMC-BAL and RAnD BAL), cells are cultured in aggregates attached to a nonwoven polyester fabric and are directly perfused with plasma.

Clinical evaluations have generally shown that treatments based on these BALs are safe and have shown that immunological reactions, zoonosis and tumorigenicity were not a problem for the patient [34, 36, 39–43]. The reported clinical studies have shown that patients may be successfully bridged to LTx with BAL-based treatents using TE liver constructs, but have not clearly proven the efficacy of treatment as compared to standard supportive treatments. The largest scale, prospective, multicenter, randomized phase II/III trial of the HepatAssist® BAL, a "first generation BAL," did show a statistically significant higher survival rate compared to controls receiving state-of-the-art standard supportive treatment, but only for patients with fulminant and subfulminant hepatic failure and only after accounting for the effect of the different etiology of ALF and liver transplantation on patients' survival [34]. Such ambiguous clinical results, the time loss of cellular activity during treatment, and the presence of a necrotic core in the cell compartment of many bioreactors at the end of treatment suggest that improvement of transport of nutrients, and metabolic wastes and products to or from the cells in the bioreactor is critical for the development of therapeutically effective BALs [44].

The knowledge and experience gained from the experimental activity in BAL development performed in the laboratory, in animal models of ALF, and in clinical evaluations indicate that the ideal bioreactor for a BAL ought to: provide cells with nutrients and oxygen; remove carbon dioxide and waste metabolites to prevent cell death; provide cells with biochemical and physical cues that foster cell reorganization into liver-like aggregates and cell differentiation; preserve the liver cell phenotype for the treatment time; prevent cell rejection (if allo- or xenogeneic cells are used) and intoxication caused by the ALF plasma; promote the unhindered transport of liver-specific metabolic products into the blood stream of the patient; and be operated so as to maximize the BAL therapeutic efficacy. Review papers are available

in literature discussing the effect of liver cell coculture, culture conditions and techniques, and cell scaffolds on bioreactor performance. In this chapter, advanced strategies that have been proposed over to improve mass transport in the bioreactors at the core of a BAL for the treatment of ALF patients are reviewed.

2 Strategies to Improve Mass Transport in BAL Bioreactors

Ensuring proper transport of essential nutrients (oxygen, sugars, amino acids, etc.) to the cells and waste metabolites (CO_2, lactate, etc.) and liver-specific metabolic products (clotting factors, growth factors, etc.) away from the cells in a TE liver construct is essential for proper bioreactor performance. Transport is, however, complicated by the high cell density typical of the natural liver tissue, typically low concentrations of nutrients, and sensitivity of liver cells to waste metabolites [26, 45–48]. Poor oxygen and glucose supply has been correlated with necrotic regions in tumors and in dense cell aggregates [49–52]. Low oxygen concentrations have been reported to affect cell viability and function [26]. Nonuniform spatial distributions of nutrients, metabolic wastes and products may have important effects on cell phenotype, motility and survival, on the bioreactor performance, and on the therapeutic efficacy of the treatment as a whole.

Conceptually, BAL bioreactors are heterogeneous (i.e., more than one phase is present) and may be thought of as being comprised of geometrically and volumetrically distinct compartments that intercommunicate through mass exchange. Membranes are often used to separate compartments in a bioreactor and their finite volume also defines them as a compartment. In fact, phenomena occurring in the membrane wall significantly affect mass exchange and overall efficacy of the bioreactor. In spite of this, BAL bioreactors are commonly classified without accounting for the membrane as a compartment – a convention we also adopt for continuity with prior literature. However, because of their importance in bioreactor performance, membranes are discussed in great detail in Sects. 2.1 and 2.3.

The RAnD BAL is a two-compartment bioreactor that radially perfuses oxygenated nutrient media (plasma in clinical operation) through a nonwoven mesh scaffold (the plasma compartment) containing adherent hepatocyte aggregates (the cell compartment). The AMC-BAL is distinguished from the RAnD BAL by axial flow rather than radial flow perfusion through a nonwoven polyester mesh scaffold containing adherent hepatocyte aggregates and a third compartment consisting of the lumen of axially aligned hydrophobic oxygenation fibers used to provide local, integral oxygen to the cells. The Arbios HepatAssist®, Excorp BLSS, and Vital Therapies ELAD® use two compartment bioreactors in which nutrient media (blood or plasma in clinical operation) flows through the lumen of hydrophilic hollow fiber membranes (first compartment) with cells housed in a second compartment external to the hollow fibers. The MELS CellModule is a four-compartment bioreactor with two independent compartments in the lumen of two distinct capillary systems bounded by hydrophilic hollow fiber membranes for nutrient media

perfusion (plasma in clinical operation), a third compartment consisting of the lumen of hydrophobic oxygenation fibers used to provide local, integral oxygen to the cells, and a fourth compartment, external to the three fiber lumen compartments, that houses cells. The hollow fiber membrane mats for media and oxygen perfusion are interposed to achieve decentralized mass exchange with low concentration gradients and scalability of the bioreactor size. The two sets of capillary media perfusion fibers can be operated in counter-directional flow, simulating "arterial" and "venous" flow in tissues.

Bioreactors are reacting systems that involve transport of nutrients and oxygen from the perfusing medium to the cells, where metabolism produces waste materials and biological products that need to be transported back to the perfusing media for removal from the bioreactor. Depending upon bioreactor construction, transport in each compartment, and through the membranes separating compartments, is either by diffusion or combined convection and diffusion. The trend in BAL bioreactor design evolution has been to add more compartments with specific functions to simulate better the native organ. Indeed, [53] suggests that four-compartment bioreactors are necessary to enable integral oxygenation and distributed mass exchange with low gradients typical of the liver.

Hollow fiber membranes for nutrient perfusion have perm-selective properties that reject high molecular weight (HMW) molecules (>100–250 kDa, depending upon the membrane) and are used to isolate physically the perfusate from the cell compartment in order to mitigate the potential of either host (patient) vs graft (cells) rejection or graft vs host reaction and, in the case of porcine liver cells, prevent the transmission of xenogeneic disease such as porcine endogenous retrovirus. Use of perfusate hollow fiber membranes, however, introduces a resistance to desirable mass transport of nutrients and metabolites that needs to be considered in bioreactor design. Because oxygen transport to and consumption by liver cells has historically been considered to be a limiting feature in liver cell culture and maintenance, hollow fiber oxygenation membranes are used by the MELS CellModule and AMC-BAL to maintain local oxygen concentrations at a relatively constant level throughout the cell compartment.

Diffusion is often the main transport mechanism for low molecular weight (LMW) solutes, also in the presence of significant net transport of fluids across compartments (i.e., convection). Convection may significantly improve transport across compartments of HMW solutes (e.g., clotting proteins or growth factors), or protein-bound hydrophobic species, that may have important effects on cell behavior or the therapeutic BAL efficacy but whose diffusivity is much smaller than LMW solutes. The spatial profile of soluble nutrients and wastes, and the rate at which they are transferred across compartments, depends on the mass transport resistance of each compartment and their concentration in the compartment where they are supplied.

Each compartment, including membrane walls, in a BAL bioreactor can be described by the equations of motion coupled with mass transport (convective and diffusive) in a reacting system. The individual compartments are coupled through matching fluxes and species concentrations at the compartment boundaries. The

following sections present and discuss some of the advanced strategies proposed to enhance transport and liver cell metabolic activity in BAL bioreactors. Transport in the oxygenation membrane compartment, when present, is not discussed because resistance in this compartment is generally negligible.

2.1 Blood/Plasma Compartment

A still unresolved question in bioreactor design for extracorporeal BALs is whether perfusion by whole blood or plasma, continuously separated from the blood using a plasma separator such as a continuous centrifuge or plasmapheresis membrane module, is preferable [54]. In both cases, plasma is the carrier for soluble and protein-bound solutes into the bioreactor and liver-specific proteins and soluble factors such as clotting factors from the bioreactor. In whole blood, the red blood cells also act as efficient oxygen and carbon dioxide transporters. In both cases, in long term extracorporeal support, even with anticoagulant supplementation (e.g., heparin or citrate), proteins in the plasma (at least those of the complement cascade) may adhere to membrane surfaces in the plasma filter and/or the bioreactor, resulting in fouling and crippling of mass exchange and separation properties. Whole blood perfusion carries the additional risk that activation of the coagulation cascade may lead to platelet cell aggregation and blood clots that totally obstruct bioreactor perfusion.

Thus, bioreactor perfusion with either blood or plasma has both advantages and disadvantages. In the following, reference is made to plasma perfusion in the blood/plasma compartment. In fact, most proposed bioreactors process plasma that is continuously separated from the patient's blood by plasmapheresis or continuous centrifugation and that generally flows along the membrane length. Similar considerations apply to culture medium and blood, but for the higher capacity of blood to carry oxygen and the effects of the possible activation of the coagulation system. However, when comparing the clinical therapeutic efficacy of BALs it should be born in mind that, even though the same blood flow rate (e.g., 100–300 mL min^{-1}) is fed to the BALs, bioreactors based on a different technology actually treat a rather different fraction of the patient's plasma volume per unit time. In fact, in plasma treating bioreactors plasma is continuously removed from the blood in the extracorporeal loop and fed to the bioreactor at flow rates that do not generally exceed 20–60 mL min^{-1}, whereas bioreactors fed with whole blood treat a plasma flow rate about three times higher.

The resistance to solute transport from the bulk plasma to the membrane surface in reactors that use membranes to separate the cell compartment from the plasma compartment or from the bulk plasma to the cell construct surface in plasma perfused bioreactors is generally lumped in a thin stagnant liquid film adjacent to the inner membrane surface or the cell construct. In the absence of significant net convective mass transport across the membrane or the construct surface, its actual value is estimated in terms of reciprocal mass transport coefficient k_c (i.e., the solute

conductivity) from nondimensional semi-empirical equations correlating the Sherwood number $Sh = k_c dD^{-1}$ with powers of nondimensional groups such as the Reynolds $Re = \rho U d \mu^{-1}$ and the Schmidt number $Sc = \mu r^{-1} D^{-1}$ (where ρ and μ are the plasma density and viscosity, respectively; U is the plasma velocity when the whole cross-sectional area is available for transport; d is the cell construct or membrane inner diameter; and D is the solute diffusivity in plasma) such as $Sh \alpha Re^{\beta} Sc^{\gamma}$ [55]. The actual type of correlation and the value of the exponents depend on the channel and construct geometry. For flow in a nonporous cylindrical tube $\beta = \gamma = 1/3$ and Sh also depends on the 1/3 power of the membrane shape ratio d/L and the 0.14 power of the viscosity ratio at the wall and in the bulk. These correlations suggest that k_c increases with the β-th power of increasing plasma velocities and the reciprocal $(1 - \beta)$-th power of d.

The occurrence of secondary flows promoted by mechanical stirring (e.g., as in a Couette flat-sheet membrane module) or by the tortuous flow around cell constructs or aggregates, or obstacles in the flow channel (as in perfused cell bioreactors or in the MELS bioreactor when operated in perfusion mode, respectively) effectively mixes the plasma and causes k_c to increase with the liquid velocity more than when plasma flows in a cylindrical tube in laminar regime [56, 57]. For this reason, in recent years the rate at which plasma is circulated through the bioreactor in BALs has been kept fairly high, at values ranging from 50–400 to 100–300 mL min^{-1} for plasma and blood, respectively, depending on bioreactor geometry.

In membrane-compartmentalized cell bioreactors with a closed shell and equipped with permeable microfiltration membranes, operation at high linear plasma velocity results in increased axial pressure drops that enhance the occurrence of filtration-reabsorption flows (i.e., Starling flows) directed from the blood compartment towards the cell compartment at the bioreactor entrance and in the opposite direction at the exit. In fact, when the bioreactor shell is closed, higher pressure in the membrane lumen than in the shell at the bioreactor entrance drives convection of plasma across the membrane wall towards the cell compartment. As the pressure in the lumen drops along the membrane axis, it eventually becomes lower than that in the shell, and fluid is returned by convection to the lumen.

Brotherton and Chau [58] have nicely shown that Starling flows enhance mass transport towards and away from the cells to a significant extent only when cell density is low. This is the case when cells are seeded at low density in the bioreactor (as in the Arbios HepatAssist® BAL), or at the beginning of culture in bioreactors seeded with immortalized cell lines (as in the Vital Therapies ELAD® BAL). At cell densities approaching that of the liver (i.e., 10^8–10^9 cells mL^{-1}), the hydraulic resistance of the cell compartment is so high as to prevent significant Starling flows from occurring. Under these conditions, operation at high linear plasma velocity (i.e., high recirculation flow rates) reduces both the solute residence time in the blood compartment and the axial nutrient concentration gradient along the bioreactor length. However, even so, cells in the cell compartment may still be functioning under a diffusion-limited regime. In bioreactors that directly perfuse plasma through the cell compartment, such as the RAnD BAL and AMC-BAL, shear-sensitive liver cells are in direct contact with the plasma and are not protected by the membrane present in

the other hollow fiber based perfusion bioreactors. In this case, the maximal linear plasma velocity is limited by the shear forces that cells may tolerate without being damaged or torn away from the construct to which they adhere.

Optimization of transport and distribution of species, such as nutrients, in the plasma perfusion compartment, and the bioreactor as a whole, may have profound effects on cell behavior and the bioreactor performance. In most bioreactors proposed for BALs, plasma is generally assumed to distribute according to ideal plug flow patterns. Hence, plasma is assumed to be thoroughly mixed over sections perpendicular to the bioreactor length and solutes in any element of fluid entering the bioreactor are all assumed to have the same residence time. The determination of species residence time distribution (RTD) in a bioreactor by means of tracer experiments is a good statistical indicator of the actual flow pattern and mixing intensity in a given bioreactor [55]. The determination of the RTD is also an effective diagnostic tool for evidence of flow maldistribution caused by fluid channeling, the formation of stagnation regions in suboptimal bioreactor design, unexpected assembly problems, or presence of developing physical interactions in long-term operation.

Tracer experiments comparing a clinical-scale MELS CellModule, where liver cells are cultured in a 3D network between different semipermeable membranes, with a laboratory-scale flat bioreactor, where plasma or medium directly perfuses cells adherent to a collagen-coated flat substratum with oxygen delivery through oxygenation membranes placed above the cells in the plasma flow channel, have shown distinctly different RTDs for the tracer [59]. The experimental apparatus was optimized to minimize the dynamic response of the tubing and the solute sensing flow-through probes in order to challenge the bioreactor with a true stepwise changing tracer concentration in the entering stream. Under these conditions the bioreactor response could be analyzed in the time domain with decreased effects of experimental error in evaluation of the bioreactor RTD. In particular, in the MELS CellModule bioreactor operated in recycle, perfusion mode at high recycle ratios R (R being the recycle-to-feed flow rate) tracer RTD was comparable to that of an ideal continuous-flow stirred tank reactor (CSTR). Reducing R caused a significant reduction of the axial mixing intensity. In the flat bioreactor, operated in single-pass mode at low feed flow rate, the tracer RTD was similar to an ideal plug flow reactor (PFR). Operation at higher feed flow rate promoted significant axial dispersion and mixing, although not as effectively as in a CSTR. Real bioreactors, in particular large clinical-scale bioreactors, rarely follow ideal flow patterns unless their design and operation is carefully developed through combination of theoretical and experimental flow modeling.

Direct cell perfusion bioreactors, such as the AMC-BAL and RAnD BAL, where liver cells adhere to the fibers of nonwoven fabrics and form aggregates with possible bridging among neighboring cellular aggregates, have potential for flow maldistribution. This occurs when cells in some regions of the bioreactor form larger, more densely packed aggregates that feature a higher hydraulic resistance to plasma flow than other regions of the bioreactor. The result is that part of the plasma will channel preferentially through the regions of low hydraulic resistance where the nutrients come in contact with the cells for shorter-than-average times. In the

regions of high hydraulic resistance, plasma comes in contact with the cells for a longer-than-average time with the potential for rapid depletion of nutrients with concomitant starving of the cells (to death). In bioreactors equipped with oxygenation membranes another cause of flow maldistribution is nonuniform membrane distribution in the plasma perfusion compartment or physical interactions between neighboring membranes in long-term operation. The formation of a segregated low flow region in the middle of a flat bioreactor caused by oxygenation membranes sticking to one another after a few hours of operation is shown in Fig. 1a. The corresponding RTD for blue dextran, shown in Fig. 1b, exhibits two separate peaks resulting from the two segregated regions in the bioreactor.

Possible causes of flow maldistribution in membrane compartmentalized cell bioreactors include nonuniform diameter of the membranes used, the deformation or occlusion of membrane lumen caused by membrane potting or cutting with worn out blades, and the formation of blood clots when the bioreactor processes whole blood.

Independent of cause, flow maldistribution generally leads to lower-than-expected biotransformation yields and may cause unpredictable distributions of cellular activities and even local cell death.

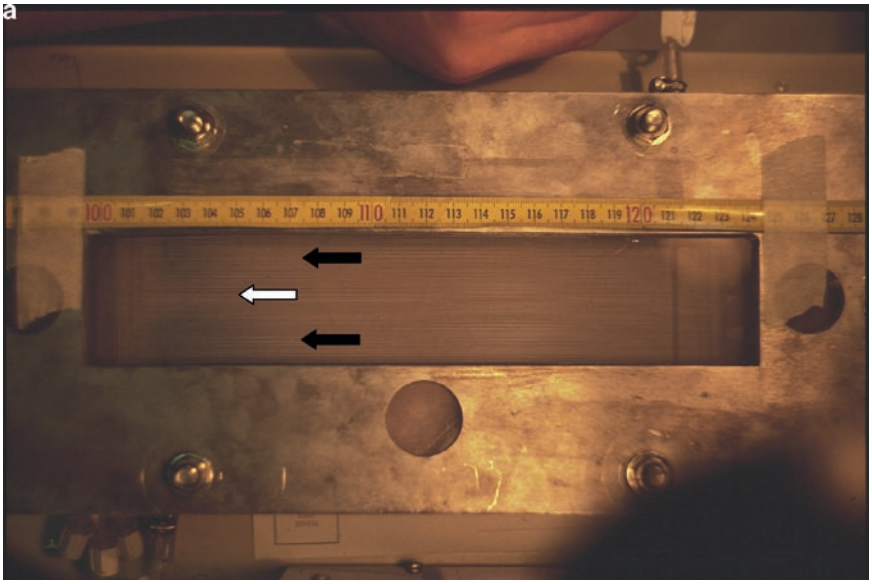

Fig. 1 **a** Photograph of tracing experiment where a flat bioreactor with oxygenation membranes hanging into the flow channel is subjected to a step challenge of blue dextran, after repeated testing for 5 h. The *black arrows* show the fast flow regions; the *white arrow* shows the low flow region in the middle of the flow channel caused by the oxygenation membranes sticking to one another. **b** RTD (*left axis, solid line*) and cumulative RTD, $F(t)$, (*right axis, open circles*) of a flat bioreactor with oxygenation membranes hanging into the flow channel after repeated testing for 5 h. The *black arrows* show the fast flow region; the *white arrow* shows the low flow region in the middle of the flow channel (see Fig. 1a) caused by the oxygenation membranes sticking to one another

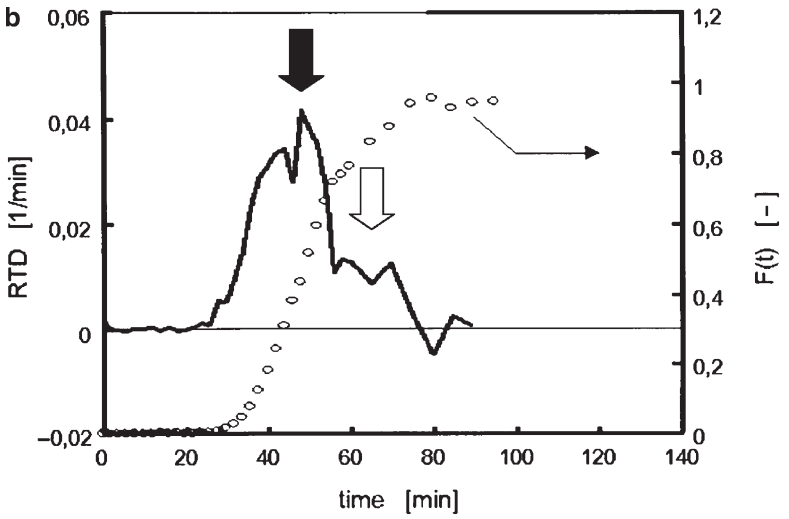

Fig. 1 (continued)

Optimization of bioreactor design and operation may minimize the detrimental effects of flow maldistribution and lead to a distribution of species that maximizes bioreactor performance and therapeutic efficacy (e.g., maintenance of cell viability and differentiated metabolic functions). In fact, in perfused cell bioreactors where species distribute in patterns similar to plug flow, metabolic products both form at rates monotonically increasing with increasing substrate concentrations and are produced by the cells more effectively than in completely mixed bioreactors (i.e., following a CSTR flow pattern). Thus, bioreactors that have plug flow characteristics require smaller cell mass for the production of a given mass of products per unit time. Likewise, larger amounts of intermediate metabolic products formed by series metabolic reactions would be produced than in completely mixed bioreactors [55].

Bioreactors featuring an established distribution of species may also be used to impose controlled gradients of oxygen, nutrients and growth factors over the cells to control their phenotype and resistance against blood-borne toxins. In fact, parenchymal liver tissue (i.e., hepatocytes) is characterized by variations of metabolic (e.g., carbohydrate metabolism) and detoxification (e.g., CYP450 enzymes) activities along the length of the sinusoid [60]. The effects of toxins and drugs have also regional specificity due to genetic and environmental cell differences [61]. This phenomenon is termed “liver zonation” and is thought to be regulated by gradients of oxygen and hormone concentrations, and extracellular matrix (ECM) composition [62, 63].

Allen and Bathia have shown that controlled steady state oxygen gradients may establish in a flat bioreactor where primary rat hepatocytes are cultured in adhesion on collagen-coated glass slides, by balancing the axial transport rate of dissolved oxygen and the cellular oxygen consumption rate (OCR) [24, 64]. They showed

that a validated transport-reaction model for the dissolved oxygen could be effectively used to adjust the bioreactor design and operation so as to establish near-physiological gradients of the dissolved oxygen concentration along the bioreactor length and avoid anoxic regions, at the same time.

In vivo, phosphoenolpyruvate carboxykinase (PEPCK) is expressed to a greater extent in periportal hepatocytes and CYP2B and CYP3A cytochrome P450 isoenzyme activities in perivenous cells. In vitro primary rat hepatocytes consistently expressed uniform PEPCK and CYP2B activities in the absence of an axial oxygen gradient. When exposed to a controlled continuous range of dissolved oxygen concentrations, the hepatocytes exhibited a heterogeneous distribution of PEPCK and CYP2B and CYP3A, when stimulated with glucagon, phenobarbital and dexamethasone respectively, mimicking their distributions in the natural liver tissue. In particular, cells in the bioreactor expressed higher PEPCK activities where they were exposed to higher dissolved oxygen concentrations, and higher CYP2B and CYP3A activities where they were exposed to lower dissolved oxygen concentrations.

Control of the axial dissolved oxygen concentration in the coculture of different liver cell types allows also for the exchange of paracrine signals among zonal subpopulations, as it occurs along the sinusoid in the natural liver. The powerful effect of physiological distributions of microenvironmental biochemical cues on CYP induction is testified by the dramatic increase reported in protein levels in the continuous-flow flat bioreactor as compared to standard Petri dish cultures challenged with the same 200 μM phenobarbital stimulus [64]. Cells exposed to a continuous range of dissolved oxygen concentrations did also respond differently when challenged with exogenous toxins similar to that happens in the natural liver [64].

Owing to the absence of red blood cells and the low solubility of O_2, the amount of O_2 carried by the plasma is much lower than that carried by the blood, and may be insufficient to meet the high OCR of hepatocytes, particularly hepatocytes attached to scaffolds [65] or exposed to toxins [66]. Supraphysiological dissolved oxygen tensions in the medium or plasma might help in meeting the hepatocyte OCR, but have been shown to compromise their viability possibly by the formation of free radicals [67, 68].

Solution to this problem was initially approached by positioning hollow fiber membranes originally developed for blood oxygenation in the plasma flow channel to replenish plasma with oxygen and remove carbon dioxide. In the first generation AMC BAL, microporous hydrophobic polypropylene hollow fiber membranes (ca. 0.6 m^2 area) were positioned as spacers in a spirally wound hydrophilic polyester nonwoven fabric, 0.4 mm thick, to supply oxygen locally [69]. In vitro tests showed evidence of anaerobic glycolytic metabolism in cells attached to the innermost regions of the fabric that was attributed to hypoxic culture conditions [70]. Recently, Mareels et al. [71] have reported on a model of momentum and oxygen transport in the space between neighboring fabric windings and into the fabric based on a commercial computational fluid dynamics (CFD) code. Simulations performed with this model confirmed that in the first generation bioreactor design only about 16–30% of the hepatocytes were adequately oxygenated. In their work, oxygenation

was considered adequate when cells could consume oxygen at 90% of their maximal uptake rate [72].

The important role of the internal membrane oxygen supply was confirmed by the fact that without internal oxygenation only a minor fraction of the cells (i.e., less then 6%) was predicted to be adequately oxygenated even when the dissolved oxygen tension in the medium entering the bioreactor was increased to ca. 300 mmHg. Model simulations also suggested that the distribution of the dissolved oxygen concentration could be enhanced by doubling the membrane packing density and/or the oxygen content of the oxygenation gas. In vitro tests on small-scale bioreactors built according to the concept used for the AMC-BAL showed that primary porcine hepatocytes cultured in enhanced bioreactor designs in which the number of oxygenation membranes had been doubled and the thickness of the fabric more than halved (e.g., 0.183 mm vs 0.4 mm) exhibited slightly improved carbohydrate metabolism and functions over the standard design but differences generally were statistically insignificant [73]. Only when a 95% oxygen gas mixture was fed to the blood oxygenation membranes, resulting in a medium dissolved oxygen tension of ca. 250 mmHg, did the cells in the enhanced bioreactor design exhibit significantly reduced anaerobic glycolytic metabolism as compared to the standard design and only after ca. 6 days of culture. Correspondingly, cells eliminated ammonia and lidocaine, and produced urea and albumin at significantly higher rates.

Another way to increase the amount of oxygen carried by plasma (during treatment), or medium (in the stand-by phase), is to add a species that reversibly binds oxygen (i.e., an oxygen carrier), which can load large amounts of oxygen from an oxygen-rich gas source and release it to the cells, as hemoglobin does in the blood. Many biocompatible oxygen carriers have been proposed as blood substitutes. Solutions of cross-linked hemoglobin (Hb) have been proposed for their oxygen-carrying capacity and long half-life time. In fact, the cross-linking stabilizes the hemoglobin molecules and prevents the breakdown of the tetramer into the a1b1 and a2b2 dimers that are toxic to the kidneys [74]. However, even after cross-linking the hemoglobins may oxidize in hours and become toxic to cells in long-term cultures [75]. Risks of xenozoonosis should also not be ruled out when xenogeneic hemoglobin is used.

To alleviate the problems associated with the use of hemoglobin, Gordon and Palmer [76] have suggested supplementation of plasma or medium with intact bovine red blood cells (bRBCs), where Hb remains confined in the cells unless they undergo lysis. Supplementation of bovine red blood cells (at ca. 10% of the human hematocrit) to the medium used for the culture of C3A hepatoma cells in the extracapillary space of a membrane compartmentalized cell reactor was reported to establish a better oxygenated cell space than in the absence of the bRBCs for up to 16 days of culture. In fact, decreased lactate production-to-glucose consumption rate ratios and increased albumin synthesis were found when the bRBCs were added to the medium. However, when primary rat hepatocytes were cultured in the same bioreactor type, the supplementation of bRBCs to the medium (at ca. 2% of the human hematocrit) was not found to improve significantly cell oxygenation in the extracapillary space [77]. A transport-reaction model of the bioreactor suggests

that this was possibly due to the higher oxygen demand of primary cells and the reduced bRBC concentration used. In any case, bRBC settling in the reservoir tank, bRBC lysis, and the formation of metoxyhemoglobin were reported to be possible problems. The latter required bRBC replacement in the medium after a few days of culture [76].

Alternatively, hemoglobin encapsulation in polyethylene glycol-decorated phospholipids bilayers has been proposed to prevent its direct contact with cells or tissue [78]. Culture of human hepatoma HepG2 cells in the presence of liposome-encapsulated hemoglobin (LEH) was reported to be toxic and inhibit cell growth [79]. This was blamed on the cell capacity to take up lipoproteins and other lipids thus causing disruption of the microcapsule lipid bilayer, and the release of toxic free hemoglobin. A recent investigation shows that addition of 20% LEH by volume to the medium used for the short-term culture (i.e., 24 h) of primary rat hepatocytes adherent on collagen-coated flat substrata did not cause significant changes to cell morphology, nor to the rate of albumin synthesis. When cultured in a flat-plate perfused cell bioreactor without LEH, larger amounts of the same cells adherent on collagen-coated flat substrata gradually died towards the bioreactor outlet, as demonstrated by the morphological deterioration of their nuclei and cytoplasm. Supplementation of 20 vol.% of LEH to the medium prevented cell death along the bioreactor length and resulted in higher rates of albumin synthesis [78]. Prior to the use of LEH in BALs, their long-term toxicity should still be investigated for both primary adult hepatocytes and hepatocyte progenitor cells. In particular, the latter might take up lipids from the LEH wall and release free hemoglobin in their growth phase. Perfluorocarbon- (PFC) based oxygen carriers have also been proposed as blood substitutes. In fact, emulsions of one or more PFCs exhibit much higher solubility of oxygen and carbon dioxide than aqueous solutions. PFCs are synthetic very stable molecules (e.g., polytetrafluoroethylene, PTFE), chemically and biologically inert, which reversibly bind up to about 20 times more oxygen and carbon dioxide than water [80]. PFCs are immiscible with water, and have to be emulsified with surfactants to add them to plasma or culture medium with which they form an oil-in-water (o/w) type emulsion. PFC emulsions have been shown to increase oxygen transfer and cell proliferation of bacterial cultures [81]. Increased oxygen transfer and proliferation have also been reported for the culture of mouse hybridoma cells cultured in PFC o/w emulsions with average droplet diameter of 0.2 μm [82], and of rat kidney cells cultured at the interface between PFC and culture medium [83], respectively. Recently, addition to circulating plasma of 20% perfluorooctyl bromide (PFOB), emulsified with egg yolk lecithin and repeatedly treated by high-pressure homogenization to yield a narrow droplet diameter distribution of 0.2 mm mean value, has been proposed for BAL bioreactors [80]. In fact, PFOB has a low toxicity and is rapidly eliminated by the reticulo-endothelial system, if it enters the blood circulation. Egg yolk lecithine does not cause complement activation as other surfactants, such as the poloxamers (e.g., Pluronics®), do. In the proposed BAL design, the PFOB/plasma o/w emulsion is kept flowing continuously in the circulation loop where it is oxygenated in a membrane oxygenator and then flows through a radial flow bioreactor where porcine liver cells are cultured in adhesion to polyurethane

foam. The PFOB droplets are removed by ultrafiltration from the plasma emulsion leaving the bioreactor, and the plasma is returned to the patient at the same flow rate as the feed after mixing it with the concentrated blood leaving the plasma separation unit [84]. These emulsions were reported to be stable, could be easily sterilized, and could be maintained in the concentrated state by ultrafiltration without breaking them up. The presence of PFOB was reported not to have adverse effects on liver cells. However, addition of 20% PFOB to plasma did not change significantly the metabolic activity of liver cells adherent to polyurethane foams but for a higher rate of lidocaine clearance [85].

2.2 Cell Compartment

Because oxygen is an important nutrient that appears to modulate hepatocyte viability and function [62, 63] and is consumed at a high metabolic rate, researchers have focused considerable effort in understanding and enhancing oxygen transport throughout the cell mass in the cell compartment. The natural liver has an extensive sinusoidal network that maintains maximal diffusion distances from the blood to any cell in the liver at less than about 100 mm [86]. Because oxygen transport to the cell mass in a BAL bioreactor is also primarily by diffusion, by analogy, hypoxic regions may develop in the cell mass when the diffusion distance exceeds approximately 100 mm [26]. If so, diffusion distances place a severe limitation on the cell mass that can be supported by a single oxygen-providing source at cell concentrations nearing that in vivo and thus impact the scale-up of BAL bioreactors from laboratory scale to clinical scale.

One way of approaching this problem is to integrate an internal oxygenator into the cell compartment as in the MELS CellModule [7, 10]. The modular repeating unit of the CellModule bioreactor features a mat of oxygenation hollow fiber membranes interposed between two mats of plasma perfusion polyethersulphone hollow fiber membranes where oxygen-rich plasma or medium flows. Liver cells are cultured in the extracapillary space outside and among the membranes and receive oxygen from all the neighboring membranes – oxygenation as well as plasma perfusion. This design effectively reduces oxygen transport limitations and establishes physiological dissolved oxygen concentration gradients across the cell mass to an extent that depends on the oxygen partial pressure in the oxygenation gas flowing in the blood oxygenation membranes, the membrane packing density and the occurrence of plasma (or medium) filtrate perfusion across the cell mass. Consequently, CellModule bioreactors have been shown to support culture of porcine and human liver cells at in vivo concentrations [17, 36] and provide metabolic synthesis and detoxification activity [87].

Some BAL bioreactor designs use protein (e.g., type I collagen) or polysaccharide (e.g., alginate) gels to replace the natural ECM and provide the hepatocytes with three-dimensional scaffolding. Use of such matrix gels has been shown to enhance attachment and to promote polarization and differentiation of primary

hepatocytes. Drawbacks to the use of gels include lowered oxygen diffusivity relative to plasma or media and a likely increase in hydraulic resistance in the cell compartment that can hinder the occurrence of Starling flow [58]. Techniques have been proposed to enhance the oxygen transport capacity of ECM substitutes either by creating micropathways to induce some degree of convective oxygen transport or by adding oxygen carriers to the gel. A transport-enhanced ECM substitute was engineered by McClelland and Coger [88–90] that incorporated porous and hollow polystyrene microspheres (0.55 μm in diameter) into a collagen type I gel. The presence of the hydrophobic microspheres was shown by confocal microscopy to form a gap between the surface of each microsphere and the surrounding hydrophilic gel material [89] through which gaseous oxygen may be transported and may proceed through the pores of the microspheres. In fact, the gap thickness is estimated to be 10 Å larger than the 2.92 Å diameter of the oxygen molecule. The higher diffusivity of oxygen through the hollow microspheres than in the gel may also be expected to contribute the enhanced transport by augmenting the effective oxygen diffusivity in the transport-enhanced ECM substitute. In both cases, the extent of transport enhancement is expected to increase with the volumetric fraction of microspheres added to the gel.

The use of the transport-enhanced ECM substitute to entrap primary rat hepatocytes (at concentrations of the order of 10^6 cells mL^{-1}) was shown to increase the oxygen transport distance from the source from approximately 170 mm or less, in the absence of microspheres, to approximately 360 and 418 mm in the presence of 20 and 40 μL microspheres per mL of collagen solution, respectively [91]. Correspondingly, a larger fraction of the cells entrapped in the transport-enhanced ECM substitute farther from the oxygen source was viable and produced urea and albumin at higher specific metabolic rates than in a normal type I collagen gel [88]. Entrapment in the transport-enhanced ECM substitute was also shown to protect effectively the cells from exposure to hypoxia and hyperoxia [91].

Another way of approaching the problem of adequate oxygenation is to add an oxygenated PFC emulsion to a type I collagen gel [92]. A 60 wt% PFC emulsion, with an average 300 nm droplet diameter and stable for at least 75 days, was prepared by dissolving Perflubron (a commercially available PFC product) in an emulsion containing egg-yolk phospholipids, followed by ultrasonication. The resulting PFC-containing ECM substitute was prepared by mixing two parts type I collagen gelling solution with one part PFC emulsion on ice while bubbling with pure oxygen. Incubation at 37 °C for 30 min produced the final PFC-containing gel. The oxygen carrier included in the collagen gel is expected to increase the oxygen supply to adherent or embedded cells. Presumably, the carrier will initially release a bolus of the oxygen stored in the gel, which may be useful during cell attachment and, spreading when oxygen demand is highest. A long-term steady state follows where oxygen diffusion is believed to be enhanced by the presence of the oxygen carrier in the gel. Indeed, a culture of primary rat hepatocytes in adhesion on the PFC-containing gel was reported to have increased hepatocyte viability, cytochrome P450 activity, albumin secretion and urea production. More noticeably, rat hepatocytes embedded in the PFC-containing gel, and cultured in standard Petri dishes, secreted albumin

at rates that continuously increased over 8 days and that, at the end of culture, were approximately 350% and 166% higher than in adhesion culture on collagen in the absence and in the presence of serum, respectively. The long-term specific urea production rate of cells embedded in the PFC-containing gel was also approximately 76–79% higher than in adhesion on collagen. However, in all cases urea was produced at rates that continuously decreased in time with a residual 20–25% urea production rate after 8 days of culture. It is worth noticing that both oxygen transport enhancement techniques can be adapted to any BAL design where cells are embedded in a gel.

2.3 Membranes

All but one of the BAL bioreactors listed in Table 1 use perm-selective membranes to segregate the various compartments of the bioreactor. Their presence is seldom accounted for in the bioreactor design in spite of the fact that membrane volume accounts for approximately 15–20% of the bioreactor volume, based on the typical membrane diameter and wall thickness used for BALs, and that the mechanism of solute transport and its interactions with the membrane material may condition the bioreactor performance.

A primary purpose of the membrane separating the blood/plasma compartment from the cell compartment is immunologic: the membrane serves to isolate the cells from direct contact with the plasma in order to prevent both host-vs-graft and graft-vs-host reactions. Experience has demonstrated that ultrafiltration membranes that reject 90+% of solutes of molecular weight greater than about 70 kDa (i.e., membranes with a nominal molecular weight cut-off of about 70 kDa) and microfiltration membranes with maximal pore size of about 0.15 μm can effectively shield cells in the bioreactor from rejection. Such membranes also reduce the risk of xenozoonosis transmission (e.g., porcine endogenous retrovirus) to the patient when xenogeneic (porcine) cells are used [41, 93–95].

The transport and separation properties of membranes interposed between the plasma and the cell compartment influence and regulate the transport of water and soluble nutrients from the plasma to the cells and products and waste metabolites from the cells to the plasma.

Elegant analyses of convective-diffusive transport across such membranes have been presented [96–98] that provide the basis for understanding how the morphology of the membrane wall and membrane physical–chemical properties affect transport across the membranes. Initial BAL approaches used commercially available cellulose acetate dialysis membranes with low nominal molecular weight cut-off (hence, good barrier properties) and low hydraulic permeability that were approved by governmental agencies for use in medical treatments. Increasing awareness of the importance of membrane transport properties in bioreactor performance led to the use of membranes with as high a hydraulic permeability as possible provided that they exhibit the necessary separation properties to ensure protection of the cellular

graft. More recent versions of BAL bioreactors use highly permeable asymmetric ultrafiltration (i.e., hemo(dia)filtration) membranes with nominal molecular weight cut-off equal to or greater than about 100 kDa, e.g., polyethersulphone – MELS CellModule [35], and microfiltration (plasmapheresis) membranes with maximal pore size of about 0.2 mm, e.g., polysulphone – Arbios HepatAssist® BAL [94, 99]. At a given axial pressure drop, highly water permeable membranes are expected to provide higher Starling flows between the blood and the cell compartment with enhanced transport of mid-to-high MW nutrients and products towards and away from the cells. However, only bioreactors operating at low cell density would see improvements in Starling flow with more highly permeable membranes. At near in vivo cell densities, highly permeable membranes do not enhance transport across compartments to any significant extent.

Membrane composition is another important factor to consider in BAL bioreactor development. Many of the membranes used thus far consist of a hydrophobic polymeric backbone that is hydrophilized by chemical attachment of hydrophilic pendant moieties or by blending with hydrophilic polymers (as in the case of most commercial polysulphone membranes) or by physical treatment (as in the case of polypropylene membranes) [100]. Only a few BAL bioreactors use commercial membranes made of hydrophilic polymers (e.g., cellulose and its derivatives) with a nominal molecular weight cut-off of about 100 kDa.

Membranes themselves are but inert selective barriers, and soluble species with hydrophobic domains tend to adsorb on the hydrophobic polymeric backbone. Adsorption of mid-to-high MW proteins, greater than 5 kDa, and/or protein-bound solutes on the plasma/blood contacting membrane surface or on the pore surface into the membrane wall are not generally accounted for in transport models, but may significantly affect the bioreactor performance. In fact, in high-flux dialysis, hemofiltration or hemodiafiltration processes, adsorption of β2-microglobulin on polymethylmethacrylate or polyacrylonitrile membranes has been reported to increase significantly clearance of β2-microglobulin from the blood of uremic patients [101]. In similar fashion, membrane adsorption of hydrophobic hepatic toxins could transiently reduce the toxin concentration and exert a protective effect on the liver cells in the bioreactor. Adsorption of immune-competent proteins could also add to the membrane separation properties to protect the cellular graft from rejection. Adsorption has also been shown to have quantitative effects on lidocaine clearance in MELS CellModule-type bioreactors without cells in the cell compartment [87].

The downside of adsorption is that liver-specific protein products or growth factors might also be adsorbed on the membrane or be rejected by membranes whose pore size has been reduced by adsorption of mid-to-high MW proteins (a phenomenon termed fouling). In fact, the nominal molecular weight cut-off of polysulphone ultrafiltration membranes was shown to decrease significantly after contacting the blood for the adsorption of plasma proteins [102]. Protein adsorption on microfiltration symmetric membranes with a hydrophobic polymeric backbone was also shown to cause a dramatic reduction of the membrane water permeability [103]. Under these conditions, the actual concentration of growth factors in the cell

compartment may be much lower than in the blood compartment (and possibly be ineffective on cell behavior). Liver-specific products produced by the cells might also not be able to cross the membrane wall and reach the patient's blood circulation; accumulation in the cell compartment might possibly compromise the potential therapeutic efficacy of metabolically active cells.

Membranes separating the blood/plasma compartment from the cell compartment can also act as an attachment surface for attachment-dependent cells in the cell compartment. This has spurred research aimed at understanding the effect of membrane surface properties on liver cell metabolism. As nicely reviewed in Legallais et al. [28], several investigators have reported on the effect on liver cell adhesion and metabolism of the membrane polymeric material [104–108], surface wettability (i.e., hydrophilicity) [109], and roughness [110]. The reported results are qualitative and rather ambiguous. For instance, physically hydrophilized (i.e., wettable) polypropylene membranes appear to favor cell adhesion and metabolic activity [109], but Cuprophan® membranes made of highly hydrophilic regenerated cellulose were not reported to perform as well as membranes with a hydrophobic backbone [105]. This is possibly due to the fact that tests were generally performed in Petri dishes under time-varying and largely uncontrolled culture conditions. The ambiguous results may also be due the fact that many other chemical–physical surface properties known to affect cell behavior, such as the type and number of functional groups, the charge, the presence of crystalline regions, the surface roughness, among others, were varied (without real control) at the same time as membrane surface hydrophilicity.

Catapano et al. [111] proposed a technique to investigate the effect of surface wettability on liver cell metabolism by using membranes of a given polymer and surface roughness, physically modified to exhibit different amounts of oxygen at the surface, while minimizing the presence of different functional groups at the membrane surface. Liver cells were cultured in adhesion on membranes in a recycle bioreactor designed and operated to culture cells at steady, uniform and measurable concentrations of soluble species [109, 112]. Under these conditions, cells consistently expressed higher metabolic activities (e.g., cells consumed oxygen at higher rates) on more wettable membranes. Moreover, cells cultured on collagen were far more active than on uncoated membranes of similar wettability, possibly because of the presence of specific amino-acid sequences in the collagen. These preliminary results suggest that, when a significant fraction of cells are in direct contact with the membranes, the chemical–physical surface properties of the membrane may have quantitative effects on cell metabolic activities and on the transport of soluble metabolites in the bioreactor. In fact, when nutrients and oxygen supplied trough the membranes are consumed at a high rate by adherent cells on the membranes their concentration may be reduced so much as to starve the cells farther away from the membrane–blood interface. Thus, the advantages of using membranes with surface chemical–physical properties favoring cell metabolism (for the polymer of which they are made or because coated with natural protein substrata, such as collagen or Matrigel®) in clinical-scale bioreactors using 3D liver constructs might even be off-set by the increased diffusional nutrient limitations that they cause. In this respect, the quantitative

characterization of membrane effects on cell metabolic reactions could provide important information to optimize the bioreactor design and operation.

The presence of plasmapheresis (plasma filter) membranes is not generally accounted for in the evaluation of perfused cell bioreactors that operate with plasma. Such membranes are often used upstream from the bioreactor to continuously separate plasma for perfusion through the bioreactor from the blood stream. Because of significant, pressure-driven separation (microfiltration) of plasma from the blood, plasma filter membranes operate under more demanding conditions than membranes inside a bioreactor. As with membranes housed inside bioreactors, plasma filter membranes are subject to fouling not only from large molecular weight species but also from cellular deposition and clot formation. Accumulation of rejected or partially rejected large solutes at the membrane interface with the blood because of poor module design and operation may cause the permeate plasma flow rate to drop to levels unacceptable for therapeutic purposes. Fouling may also cause plasma proteins such as albumin to be largely retained in the blood stream, indirectly hindering the detoxification function of the BAL. While plasma filters can be replaced when performance drops below acceptable levels, the result is typically hemodilution in patients with already poor coagulation capacity – an undesirable clinical event.

3 Conclusions and Perspectives

Each of the proposed enhancement techniques presented in this chapter has been shown to bring about transport enhancements, though to different degrees, that yield better bioreactor metabolic performance in the short term. However, none of them has yielded stable cell expression of most metabolic functions typical of differentiated adult hepatocytes for longer than about a week. Nor has any technique been used for large clinical-scale BALs except for the internal oxygenation membranes in the MELS CellModule. Whether success from a single technique for enhancing transport in bioreactors on the scale of milliliters will yield similar transport enhancements when scaled to bioreactors hosting hundreds of grams of liver cells avidly consuming these nutrients remains to be seen. The integration of more transport enhancement techniques in the different compartments of a large scale bioreactor is more likely to result in more consistent transport and performance enhancements.

However, a large number of papers has been published in the last few years on the effects on hepatocyte metabolism of the characteristics of the scaffold to which they attach (i.e., geometry, morphology, physical–chemical properties, patterns of immobilized biochemical cues, etc.), and the coculture of different liver cells. Both are known to affect the liver cell organization and the hepatocyte phenotype. This suggests that techniques should be developed to control in vitro the microarchitecture of the liver cells after they are seeded into, or on, a scaffold to foster their organization in in vivo-like structures and promote mass transport mechanisms mimicking those of the liver *acinus*. The impact on cell behavior of controlling

the microenvironment and the mechanisms of mass transport has been demonstrated in mL-scale bioreactors, where the microfluidic environment allows the control of nanoliter fluid volumes and flows [113]. Integration of the knowledge of the mechanisms controlling cell arrangement and motility in porous scaffolds and of the factors affecting mass transport to, and away from, dense liver cell aggregates might provide design principles to better approximate the in vivo microenvironment also in clinical-scale bioreactors for BALs.

References

1. Bernal W, Auzinger, G, Sizer, E, and Wendon, J (2008) Intensive care management of acute liver failure. Sem Liver Dis 28:188–200
2. Lee WM, Squires RH Jr, Nyberg SL, Doo E, Hoofnagle JH (2008) Acute liver failure: summary of a workshop. Hepatology 47:1401–1415
3. Hadem J, Stiefel P, Bahr MJ, Tillmann HL, Rifai K, Klempnauer J, Wedemeyer H, Manns MP, Schneider AS (2008) Prognostic implications of lactate, bilirubin, and etiology in German patients with acute liver failure. Clin Gastroenterol Hepatol 6:339–345
4. Liou IW, Larson AM (2008) Role of liver transplantation in acute liver failure. Semin Liver Dis 28:201–209
5. Mullhaupt B, Dimitroulis D, Gerlach JT, Clavien PA (2008) Hot topics in liver transplantation: organ allocation – extended criteria donor – living donor liver transplantation. J Hepatol 48(Suppl 1):S58–S67
6. Polson J, Lee WM (2007) Etiologies of acute liver failure: location, location, location! [comment]. Liver Transplant 13:1362–1363
7. Gerlach JC, Zeilinger K, Patzer JF II (2008) Bioartificial liver: why, what, whither. Regen Med 3(4):575–595
8. Blei AT (2005) Selection for acute liver failure: have we got it right? Liver Transplant 11:S30–S34
9. Lin TY, Lee CS, Chen CC, Lian KY, Lin WSJ (1979) Regeneration of human liver after hepatic lobectomy studies by repeated liver scanning and repeated needle biopsy. Ann Surg 190:48–53
10. Gerlach J, Hout M, Gage K, Zeilinger K (2008) Liver cell-based therapy – bioreactors as enabling technology. In: Principles of regenerative medicine, Elsevier, Amsterdam, pp 1086–1105
11. Gruttadauria S, Mandala L, Miraglia R, Caruso S, Minervini MI, Biondo D, Volpes R, Vizzini G, Marsh JW, Luca Aet-al. (2007) Successful treatment of small-for-size syndrome in adult-to-adult living-related liver transplantation: single center series. Clin Transplant 21:761–766
12. Matsushita M, Nose Y (1986) Artificial liver. Artif Organs 10:378–384
13. Denis J, Opolon P, Delorme ML, Granger A, Darnis F (1979) Long-term extra-corporeal assistance by continuous haemofiltration during fulminant hepatic failure. Gastroenterol Clin et Biol 3:337–347
14. Knell AJ, Dukes DC (1976) Dialysis procedures in acute liver coma. Lancet 2:402–403
15. Mitzner SR, Stange J, Klammt S, Peszynski P, Schmidt R, Noldge-Schomburg G (2001) Extracorporeal detoxification using the molecular adsorbent recirculating system for critically ill patients with liver failure. J Am Soc Nephrol 12(Suppl 17):S75–S82
16. Opolon P (1979) High-permeability membrane hemodialysis and hemofiltration in acute hepatic coma: experimental and clinical results. Artif Organs 3:354–360
17. Sauer IM, Zeilinger K, Pless G, Kardassis D, Theruvath T, Pascher A, Goetz M, Neuhaus P, Gerlach JC (2003) Extracorporeal liver support based on primary human liver cells and albumin dialysis – treatment of a patient with primary graft non-function. J Hepatol 39:649–653

18. Willinger M, Schima H, Schmidt C, Huber L, Vogt G, Falkenhagen D, Losert U (1999) Microspheres based detoxification system: in vitro study and mathematical estimation of filter performance. Int J Artif Organs 22:573–582
19. Pascher A, Sauer IM, Hammer C, Gerlach JC, Neuhaus P (2002) Extracorporeal liver perfusion as hepatic assist in acute liver failure: a review of world experience [see comment]. Xenotransplantation 9:309–324
20. Gerlach JC, Zeilinger K (2002) Adult stem cell technology – prospects for cell based therapy in regenerative medicine. Int J Artif Organs 25:83–90
21. Gerlach JC, Kloppel K, Muller C, Schnoy N, Smith MD, Neuhaus P (1993) Hepatocyte aggregate culture technique for bioreactors in hybrid liver support systems. Int J Artif Organs 16:843–846
22. Nyberg SL, Shatford RA, Hu WS, Payne WD, Cerra FB (1992) Hepatocyte culture systems for artificial liver support: implications for critical care medicine (bioartificial liver support) Crit Care Med 20:1157–1168
23. Dahmen U, Madrahimov N, Madrahimova F, Ji Y, Schenk A, Dirsch O (2008) Small-for-Size syndrome in the rat: does size or technique matter? J Surg Res 149(1):15–26
24. Allen JW, Hassanein T, Bhatia SN (2001) Advances in bioartificial liver devices. Hepatology 34:447–455
25. Busse B, Smith MD, Gerlach JC (1999) Treatment of acute liver failure: hybrid liver support. A critical overview. Langenbecks Arch Surg 384:588–599
26. Catapano G, De Bartolo L, Lombardi CP, Drioli E (1996) The effect of oxygen transport resistances on the viability and functions of isolated rat hepatocytes. Int J Artif Organs 19:61–71
27. Catapano G, Gerlach JC (2007) Bioreactors for liver tissue engineering. In: Ashammakhi N , Reis R , Chiellini E (eds.) Topics in tissue engineering, pp 1 – 41
28. Legallais C, David B, Dore E (2001) Bioartificial livers (BAL): current technological aspects and future developments. J Membr Sci 181:81–95
29. Park JK, Lee DH (2005) Bioartificial liver systems: current status and future perspective. J Biosci Bioeng 99:311–319
30. Patzer JFII (2001) Advances in bioartificial liver assist devices. Ann N Y Acad Sci 944:320–333
31. Mazariegos GV, Kramer DJ, Lopez RC, Shakil AO, Rosenbloom AJ, DeVera M, Giraldo M, Grogan TA, Zhu Y, Fulmer ML et-al. (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
32. Mazariegos GV, Patzer JF, Lopez RC, Giraldo M, deVera ME, Grogan TA, Zhu Y, Fulmer ML, Amiot BP, Kramer DJ (2002) First clinical use of a novel bioartificial liver support system (BLSS) Am J Transplant 2:260–266
33. Millis JM, Cronin DC, Johnson R, Conjeevaram H, Conlin C, Trevino S, Maguire P (2002) Initial experience with the modified extracorporeal liver-assist device for patients with fulminant hepatic failure: system modifications and clinical impact. Transplantation 74:1735–1746
34. Demetriou AA, Brown RS, Busuttil RW, Fair J, McGuire BM, Rosenthal P, Esch JSA, Lerut J, Nyberg SL, Salizzoni M et-al. (2004) Prospective, randomized, multicenter, controlled trial of a bioartificial liver in treating acute liver failure. Ann Surg 239:660–667
35. Gerlach JC, Mutig K, Sauer IM, Schrade P, Efimova E, Mieder T, Naumann G, Grunwald A, Pless G, Mas A et al. (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
36. Sauer IM, Kardassis D, Zeillinger K, Pascher A, Gruenwald A, Pless G, Irgang M, Kraemer M, Puhl G, Frank J et al. (2003) Clinical extracorporeal hybrid liver support – phase I study with primary porcine liver cells. Xenotransplantation 10:460–469
37. van de Kerkhove MP, Di Florio E, Scuderi V, Mancini A, Belli A, Bracco A, Dauri M, Tisone G, Di Nicuolo G, Amoroso P et al. (2002) Phase I clinical trial with the AMC-bioartificial liver. Int J Artif Organs 25:950–959

38. Morsiani E, Pazzi P, Puviani AC, Brogli M, Valieri L, Gorini P, Scoletta P, Marangoni E, Ragazzi R, Azzena G et-al. (2002) Early experiences with a porcine hepatocyte-based bioartificial liver in acute hepatic failure patients. Int J Artif Organs 25:192–202
39. Baquerizo A, Mhoyan A, Shirwan H, Swensson J, Busuttil RW, Demetriou AA, Cramer DV (1997) Xenoantibody response of patients with severe acute liver failure exposed to porcine antigens following treatment with a bioartificial liver. Transplant Proc 29:964–965
40. Ellis AJ, Hughes RD, Wendon JA, Dunne J, Langley PG, Kelly JH, Gislason GT, Sussman NL, Williams R (1996) Pilot-controlled trial of the extracorporeal liver assist device in acute liver failure. Hepatology 24:1446–1451
41. Kuddus R, Patzer JF II, Lopez R, Mazariegos GV, Meighen B, Kramer DJ, Rao AS (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
42. Millis JM, Cronin DC, Johnson R, Conjeevaram H, Brady L, Trevino S, Conlin C, Brotherton J, Traglia D, Dane G et-al. (2001) Safety of continuous human liver support. Transplant Proc 33:1954
43. Pitkin Z, 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
44. Kamohara Y, Rozga J, Demetriou AA (1998) Artificial liver: review and Cedars-Sinai experience. J Hepato-Biliary-Pancreatic Surg 5:273–285
45. Balis UJ, Behnia K, Dwarakanath B, Bhatia SN, Sullivan SJ, Yarmush ML, Toner M (1999) Oxygen consumption characteristics of porcine hepatocytes. Metab Eng 1:49–62
46. Catapano G, De Bartolo L, Lombardi CP, Drioli E (1996) The effect of catabolite concentration on the viability and functions of isolated rat hepatocytes. Int J Artif Organs 19:245–250
47. Freshney R (2000) Culture of animal cells – a manual of basic techniques, 4th edn. Wiley, New York
48. Rotem A, Toner M, Bhatia S, Foy BD, Tompkins RG, Yarmush ML (1994) Oxygen is a factor determining in-vitro tissue assembly – effects on attachment and spreading of hepatocytes. Biotechnol Bioeng 43:654–660
49. Mueller-Klieser WF, Sutherland RM (1982) Oxygen tensions in multicell spheroids of two cell lines. Br J Cancer 45:256–264
50. Sutherland RM, Sordat B, Bamat J, Gabbert H, Bourrat B, Mueller-Klieser W (1986) Oxygenation and differentiation in multicellular spheroids of human colon carcinoma. Cancer Res 46:5320–5329
51. Tannock IF (1972) Oxygen diffusion and the distribution of cellular radiosensitivity in tumours. Br J Radiol 45:515–524
52. Vaupel P (1977) Hypoxia in neoplastic tissue. Microvasc Res 13:399–408
53. Xu ASL, Luntz TL, Macdonald JM, Kubota H, Hsu E, London RE, Reid LM (1999) Lineage and biology and liver. In: Lanza RP, Langer R, Vacanti J (eds.) Principles of tissue engineering, 2nd edn. San Diego, Academic Press, chap. 41, pp 559–598
54. Patzer JF II, Campbell B, Miller R (2002) Plasma versus whole blood perfusion in a bioartificial liver assist device. ASAIO J 48:226–233
55. Levenspiel O (1999) Chemical Reaction Engineering, 3rd edn. Wiley, New York
56. Catapano G, Papenfuss HD, Wodetzki A, Baurmeister U (2001) Mass and momentum transport in extra-luminal flow (ELF) membrane devices for blood oxygenation. J Membr Sci 184:123–135
57. Ozisik NM (1985) Heat Transfer. McGraw-Hill, New York
58. Brotherton JD, Chau PC (1996) Modeling of axial-flow hollow fiber cell culture bioreactors. Biotechnol Prog 12:575–590
59. Catapano G, Euler M, Gaylor JDS, Gerlach J (2001) Characterization of the distribution of matter in hybrid liver support devices where cells are cultured in a 3D membrane network or on flat substrata. Int J Artif Organs 24:102–109
60. Jungermann K, Thurman RG (1992) Hepatocyte heterogeneity in the metabolism of carbohydrates. Enzyme 46:33–58

61. Lindros KO (1997) Zonation of cytochrome P450 expression, drug metabolism and toxicity in liver. General Pharmacol 28:191–196
62. Jungermann K, Kietzmann T (2000) Oxygen: modulator of metabolic zonation and disease of the liver. Hepatology 31:255–260
63. Kietzmann T, Jungermann K (1997) Modulation by oxygen of zonal gene expression in liver studied in primary rat hepatocyte cultures. Cell Biol Toxicol 13:243–255
64. Allen JW, Khetani SR, Bhatia SN (2005) In vitro zonation and toxicity in a hepatocyte bioreactor. Toxicol Sci 84:110–119
65. Foy BD, Rotem A, Toner M, Tompkins RG, Yarmush ML (1994) A device to measure the oxygen uptake rate of attached cells: importance in bioartificial organ design. Cell Transplant 3:515–527
66. Catapano G, De Bartolo L (2002) Combined effect of oxygen and ammonia on the kinetics of ammonia elimination and oxygen consumption of adherent rat liver cells. Int J Artif Organs 25(2):151–157
67. Fariss MW (1990) Oxygen toxicity: unique cytoprotective properties of vitamin E succinate in hepatocytes. Free Radical Biol Med 9:333–343
68. Martin H, Sarsat JP, Lerche-Langrand C, Housset C, Balladur P, Toutain H, Albaladejo V (2002) Morphological and biochemical integrity of human liver slices in long-term culture: effects of oxygen tension. Cell Biol Toxicol 18:73–85
69. Flendrig LM, la Soe JW, Jorning GG, Steenbeek A, Karlsen OT, Bovee WM, Ladiges NC, te Velde AA, Chamuleau RA (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
70. van de Kerkhove MP, Poyck PP, van Wijk AC, Galavotti D, Hoekstra R, van Gulik TM, Chamuleau RA (2005) Assessment and improvement of liver specific function of the AMC-bioartificial liver. Int J Artif Organs 28:617–630
71. Mareels G, Poyck PP, Eloot S, Chamuleau RA, Verdonck PR (2006) Three-dimensional numerical modeling and computational fluid dynamics simulations to analyze and improve oxygen availability in the AMC bioartificial liver. Ann Biomed Eng 34:1729–1744
72. Patzer JF II (2004) Oxygen consumption in a hollow fiber bioartificial liver--revisited. Artif Organs 28:83–98
73. Poyck PP, Mareels G, Hoekstra R, van Wijk AC, van der Hoeven TV, van Gulik TM, Verdonck PR, Chamuleau RA (2008) Enhanced oxygen availability improves liver-specific functions of the AMC bioartificial liver. Artif Organs 32:116–126
74. Chang TM (1999) Future prospects for artificial blood. Trends Biotechnol 17:61–67
75. Alayash AI (1999) Hemoglobin-based blood substitutes: oxygen carriers, pressor agents, or oxidants? Nat Biotechnol 17:545–549
76. Gordon J, Palmer AF (2005) Impact of increased oxygen delivery via bovine red blood cell supplementation of culturing media on select metabolic and synthetic functions of C3A hepatocytes maintained within a hollow fiber bioreactor. Artif Cells Blood Subst Biotechnol 33(3):297–306
77. Sullivan JP, Palmer AF (2006) Targeted oxygen delivery within hepatic hollow fiber bioreactors via supplementation of hemoglobin-based oxygen carriers. Biotechnol Prog 22:1374–1387
78. Naruto H, Huang H, Nishikawa M, Kojima N, Mizuno A, Ohta K, Sakai Y (2007) Feasibility of direct oxygenation of primary-cultured rat hepatocytes using polyethylene glycol-decorated liposome-encapsulated hemoglobin (LEH) J Biosci Bioeng 104:343–346
79. Sakai Y, Huang H, Naruto H, Nishikawa M, Kojima N, Mizuno A, Ohta K (2006) Use of liposome-encapsulated hemoglobin (LEH) as an oxygen carrier to cultured cells. In: Peppas NA, Hoffman AS, Kanamori T, Tojo K (eds.) Advances in medical engineering, drug delivery systems and therapeutic systems, American Institute of Chemical Engineering, New York, pp 45–50
80. Moolman FS (2004) Oxygen carriers for a novel bio-artificial liver support system. Pretoria, University of Pretoria

81. Lowe KC, Anthony P, Wardrop J, Davey MR, Power JB (1997) Perfluorochemicals and cell biotechnology. Artif. Cells Blood Substitutes Immobilization Biotechnol 25:261–274
82. Ju LK, Armiger WB (1992) Use of perfluorocarbon emulsions in cell culture. Biotechniques 12:258–263
83. Sanfilippo B, Ciardiello F, Salomon DS, Kidwell WR (1988) Growth of cells on a perfluorocarbon-medium interphase: a quantitative assay for anchorage-independent cell growth. In Vitro Cell Dev Biol 24:71–78
84. Van Der Merwe S, Moolman FS, Bond RP, Van Wyk AJ (2002) Bio-reactor device, PCT, Patent WO02/22775
85. Nieuwoudt MJ, Moolman SF, Van Wyk KJ, Kreft E, Olivier B, Laurens JB, Stegman FG, Vosloo J, Bond R, van der Merwe SW (2005) Hepatocyte function in a radial-flow bioreactor using a perfluorocarbon oxygen carrier. Artif Organs 29:915–918
86. Koeppen BM, Stanton BA (2008) Berne and Levy Physiology, 6th edn. Mosby, St. Louis, MO
87. Unger JK, Catapano G, Horn NA, Schroers A, Gerlach JC, Rossaint R (2000) Comparative analysis of metabolism of medium- and plasma perfused primary pig hepatocytes cultured around a 3D membrane network. Int J Artif Organs 23:104–110
88. McClelland RE, Coger RN (2000) Use of micropathways to improve oxygen transport in a hepatic system. J Biomechan Eng 122:268–273
89. McClelland RE, Coger RN (2004) Effects of enhanced O(2) transport on hepatocytes packed within a bioartificial liver device. Tissue Eng 10:253–266
90. McClelland RE, MacDonald JM, Coger RN (2003) Modeling O2 transport within engineered hepatic devices. Biotechnol Bioeng 82:12–27
91. Niu M, Clemens MG, Coger RN (2008) Optimizing normoxic conditions in liver devices using enhanced gel matrices. Biotechnol Bioeng 99:1502–1512
92. Nahmias Y, Kramvis Y, Barbe L, Casali M, Berthiaume F, Yarmush ML (2006) A novel formulation of oxygen-carrying matrix enhances liver-specific function of cultured hepatocytes. FASEB J 20:2531–2533
93. Gleissner M, Bornemann R, Stemerowicz R, Meissler M, Neuhaus P, Gerlach JC (1997) Immunoisolation of hybrid liver support systems by semipermeable membranes. Int J Artif Organs 20:644–649
94. Mullon C (1999) Bioartificial organs may help reduce risk of zoonosis in xenotransplantation. Artif Organs 23:366–367
95. Nyberg SL, Hibbs JR, Hardin JA, Germer JJ, Persing DH (1999) Transfer of porcine endogenous retrovirus across hollow fiber membranes: significance to a bioartificial liver [see comment]. Transplantation 67:1251–1255
96. Baker RW (2004) Membrane transport theory. In: Membrane technology and applications, 2nd edn. New York, Wiley, chap 2, 15–84
97. Michaels AS (1966) Operating parameters and performance criteria for hemodialyzers and other membrane-separation devices. Trans Am Soc Artif Intern Organs 12:387–392
98. Nakao S, Kinura S (1982) Models of membrane transport phenomena and their applications for ultrafiltration data. J Chem Eng Jpn 15:200
99. Watanabe FD, Mullon CJ, Hewitt WR, Arkadopoulos N, Kahaku E, Eguchi S, Khalili T, Arnaout W, Shackleton CR, Rozga J et-al. (1997) Clinical experience with a bioartificial liver in the treatment of severe liver failure. A phase I clinical trial. Ann Surg 225:484–491; discussion 491–484
100. Catapano G, Vienken J (2008) Medical applications. In: Li N , Fane AG, Ho WSW, Matsuura T (eds.) Advanced membrane science and technology, Wiley, New York
101. Clark WR, Hamburger RJ, Lysaght MJ (1999) Effect of membrane composition and structure on solute removal and biocompatibility in hemodialysis. Kidney Int 56:2005–2015
102. Feldhoff P, Turnham T, Klein E (1984) Effect of plasma proteins on the sieving spectra of hemofilters. Artif Organs 8:186–192
103. Jonsson G (1986) Transport phenomena in ultrafiltration: membrane selectivity and boundary layer phenomena. Pure Appl Chem 58:1647–1656

104. Gerlach J, Stoll P, Schnoy N, Bucherl ES (1990) Membranes as substrates for hepatocyte adhesion in liver support bioreactors. Int J Artif Organs 13:436–441
105. Gerlach JC, Schnoy N, Vienken J, Smith M, Neuhaus P (1996) Comparison of hollow fibre membranes for hepatocyte immobilisation in bioreactors. Int J Artif Organs 19:610–616
106. Jozwiak A, Karlik W, Wiechetek M, Werynski A (1998) Attachment and metabolic activity of hepatocytes cultivated on selected polymeric membranes. Int J Artif Organs 21:460–466
107. Krasteva N, Seifert B, Hopp M, Malsch G, Albrecht W, Altankov G, Groth T (2005) Membranes for biohybrid liver support: the behaviour of C3A hepatoblastoma cells is dependent on the composition of acrylonitrile copolymers. J Biomater Sci Polym Ed 16:1–22
108. Qiang S, Yaoting Y, Hongyin L, Klinkmann H (1997) Comparative evaluation of different membranes for the construction of an artificial liver support system. Int J Artif Organs 20:119–124
109. Catapano G, Di Lorenzo MC, Della Volpe C, De Bartolo L, Migliaresi C (1996) Polymeric membranes for hybrid liver support devices: the effect of membrane surface wettability on hepatocyte viability and functions. J Biomater Sci Polym Ed 7:1017–1027
110. De Bartolo L, Catapano G, Della Volpe C, Drioli E (1999) The effect of surface roughness of microporous membranes on the kinetics of oxygen consumption and ammonia elimination by adherent hepatocytes. J Biomater Sci Polym Ed 10:641–655
111. Catapano G, Speranza G, Maniglio D, De Bartolo L, Della Volpe C (2002) Bioreactor type and operating conditions influence cell response to polymeric material properties. Proceedings of the IEEE-EMBS Special Topic Conference on “Molecular, Cellular and Tissue Engineering”, Genova (Italy), 6–9 June 2002
112. Catapano G, De Bartolo L (1998) Technique for the kinetic characterization of the metabolic reactions of hepatocytes in adhesion culture. Biotechnol Prog 14:500–507
113. Walker GM, Zeringue HC, Beebe DJ (2004) Microenvironment design considerations for cellular scale studies. Lab on a Chip 4:91–97