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Review Article

Hepatocyte hollow-fibre bioreactors: design, set-up, validation and applications

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Abstract

Hepatocytes carry out many vital biological functions, such as synthetic and catabolic reactions, detoxification and excretion. Due to their ability to restore a tissue-like environment, hollow-fibre bioreactors (HFBs) show great potential among the different systems used to culture hepatocytes. Several designs of HFBs have been proposed in which hepatocytes or hepatocyte-derived cell lines can be cultured in suspensions or on a solid support. Currently the major use of hepatocyte HFBs is as bioartificial livers to sustain patients suffering from acute liver failure, but they can also be used to synthesize cell products and as cellular models for drug metabolism and transport studies. Here, we present an overview of the set-up of hepatocyte HFBs and aim to provide potential users with the basic knowledge necessary to develop their own system. First, general information on HFBs is given, including basic principles, transport phenomena, designs and cell culture conditions. The importance of the tests necessary to assess the performance of the HFBs, i.e. the viability and functionality of hepatocytes, is underlined. Special attention is paid to drug metabolism studies and to adequate analytical methods. Finally, the potential uses of hepatocyte HFBs are described.

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Dedication: This review is dedicated to the memory of Dr Joachim M. Mayer, who was the initiator and, until his untimely death, supervisor of our HFB project

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Introduction

As one of the most important organs in the body, the liver carries out a multitude of functions essential to the survival of the organism. First, the liver is the main organ of lipid metabolism and particularly of β-oxidation. Second, it plays a role in glucide metabolism, converting galactose and fructose to the essential glucose. Third, it is active in the metabolism of proteins, including urea formation to eliminate the neurotoxic ammonia produced during protein catabolism. It also has a secretory function and synthesizes bile acids for fat digestion and many plasma proteins, such as clotting factors and albumin. Finally, the liver plays a key role in the catabolism of endogenous and exogenous substances, and facilitates their excretion. These functions are carried out by the hepatocytes, namely the epithelial cells that constitute 80% of the liver volume.

Hepatocytes have been used extensively as in-vitro models to investigate the above processes. Methods routinely used for hepatocyte culture include suspensions, adhesion on solid support, and more recently hollow-fibre bioreactors (HFBs). A hepatocyte HFB consists of a biological element (the hepatocytes) and a synthetic bioreactor made of a plastic shell and semi-permeable hollow fibres (HFs). By allowing a dynamic three-dimensional mode of cell culture, HFBs restore a tissue-like cell density and allow interactions between cells, simulating physiological conditions. HFBs have been used with different cell types to generate cell products such as antibodies or hormones (Tharakan et al 1988), and as artificial organs such as bioartificial livers (BALs), pancreas, kidneys and parathyroid glands (Nyberg et al 1993a). In recent years, HFBs have been used increasingly as BALs to detoxify the blood of patients with acute liver failure (ALF) (Allen et al 2001).

When using a hepatocyte HFB as a liver model, one must verify its viability and functionality, in particular that it retains the capacity to carry out essential hepatic processes such as anabolic and catabolic functions, detoxification and excretion. The use of marker compounds to evaluate the viability and functionality of hepatocytes is thus of the utmost importance.

Principles and designs of hepatocyte HFBs

Principles

An HFB consists of a network of HFs bundled together within a transparent plastic shell that has two compartments: the extracapillary space (ECS) between the HF and the plastic shell, and the intracapillary space (ICS) inside the HF (Figure 1). The first system described in the literature (Knazek et al 1972) is made of a reservoir for the culture medium, a pump, an oxygenation system and an HFB. The perfusion solution is continually pumped from a reservoir, loaded with O_2 and CO_2 through silicone tubing, driven along the HFs and radially through the semi-permeable membrane into the ECS, and returned back to the reservoir. Under standard conditions, the entire system is placed in a CO_2 incubator at 37 °C. Although different HFB configurations have been developed, the dynamic features of the perfusion system remain similar. These conditions simulate the physiological environment, and the HFB behaves like an organ. The HFs act as blood vessels, the culture medium (oxygenated by an artificial lung) replaces the blood, and the blood circulation is maintained by a pump acting as an artificial heart.

Solute transport phenomena in HFBs

Transport phenomena are of the utmost biological significance since they directly affect cell viability and functionality, and hence the performance of the HFBs. The fluid circulating within the HFs brings O_2 and nutrients to cells and removes CO_2 and other waste products. Transport phenomena depend on the design of the HFB and on culture conditions.

In an HFB, one can distinguish at least three regions (Figure 2): (i) the medium (or blood/plasma in the case of BALs) compartment where the culture medium (or blood/plasma) flows, (ii) the membrane compartment and (iii) the cell compartment, where cells are located (Catapano 1996).

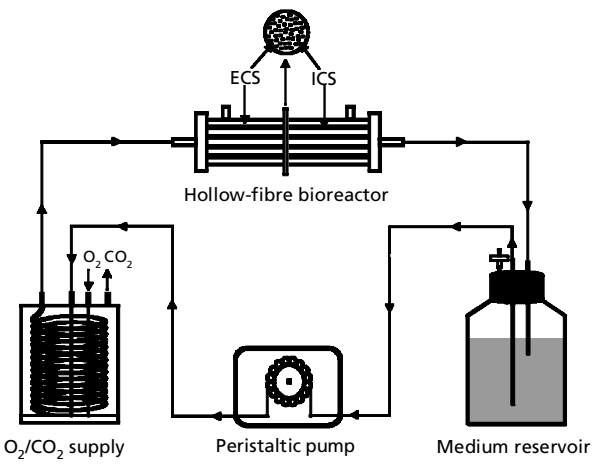

Figure 1 Schematic illustration of an HFB perfusion system. The culture medium, while continuously pumped from a reservoir and charged with O_2 and CO_2, flows into the HFs, diffuses radially through the semi-permeable membrane into the ECS and returns to the reservoir.

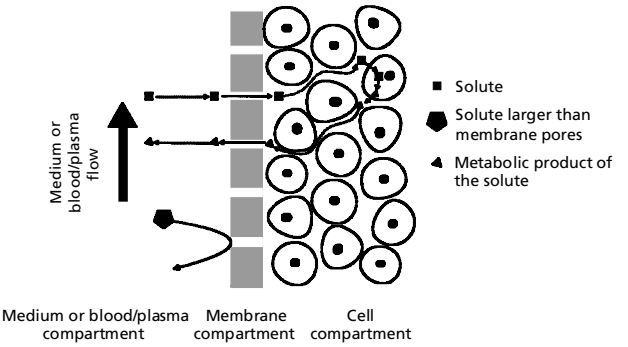

Figure 2 Mass transport phenomena in HFBs. Membrane porosity is an important characteristic of HFBs as it determines the size of the solutes able to permeate across the membrane.

In order to permeate from the culture medium to the cells, O_2 and nutrients must cross the three compartments, namely:

1. Migration from the bulk medium to the medium–membrane interface, which generally occurs according to a diffusion–convection mechanism.
2. Permeation across the semi-permeable membranes, which occurs by diffusion and/or convection in response to existing trans-membrane concentration or pressure gradients, respectively. The porosity and the surface of the HFs, as well as the membrane thickness, are important parameters that influence transfer. Increasing pore size increases solute transfer by both diffusion and convection, but also facilitates transport of immunogenic proteins in the case of BALs. Membrane porosity is therefore an important characteristic of HFBs as it determines the size of permeating solutes.
3. Migration from the membrane surface through the cell mass, which is limited by the presence of cells in the cell compartment. Collagen and other extracellular matrix proteins often used to provide a substratum for cell attachment may also decrease O_2 and solute mobility (Nyberg et al 1991).

In contrast to HFBs, transport in the liver is achieved primarily by convection along the sinusoid with a short diffusion distance ($< 5\,\mu$m) across the Disse space (Allen et al 2001).

Designs of HFBs

Various HFB configurations have been used for cell cultures (Figure 3). We distinguish here between single circuits where the HFB is perfused with only one flux of culture medium (or the blood/plasma in the case of BALs), and multiple circuits with two or more fluxes.

Single-circuit systems. The simple mode (Figure 3A) corresponds to the first model described by Knazek et al (1972). Cells are cultured outside the HFs, while the culture medium is perfused inside. This is the simplest and still most common system in use. In this model, pressure in

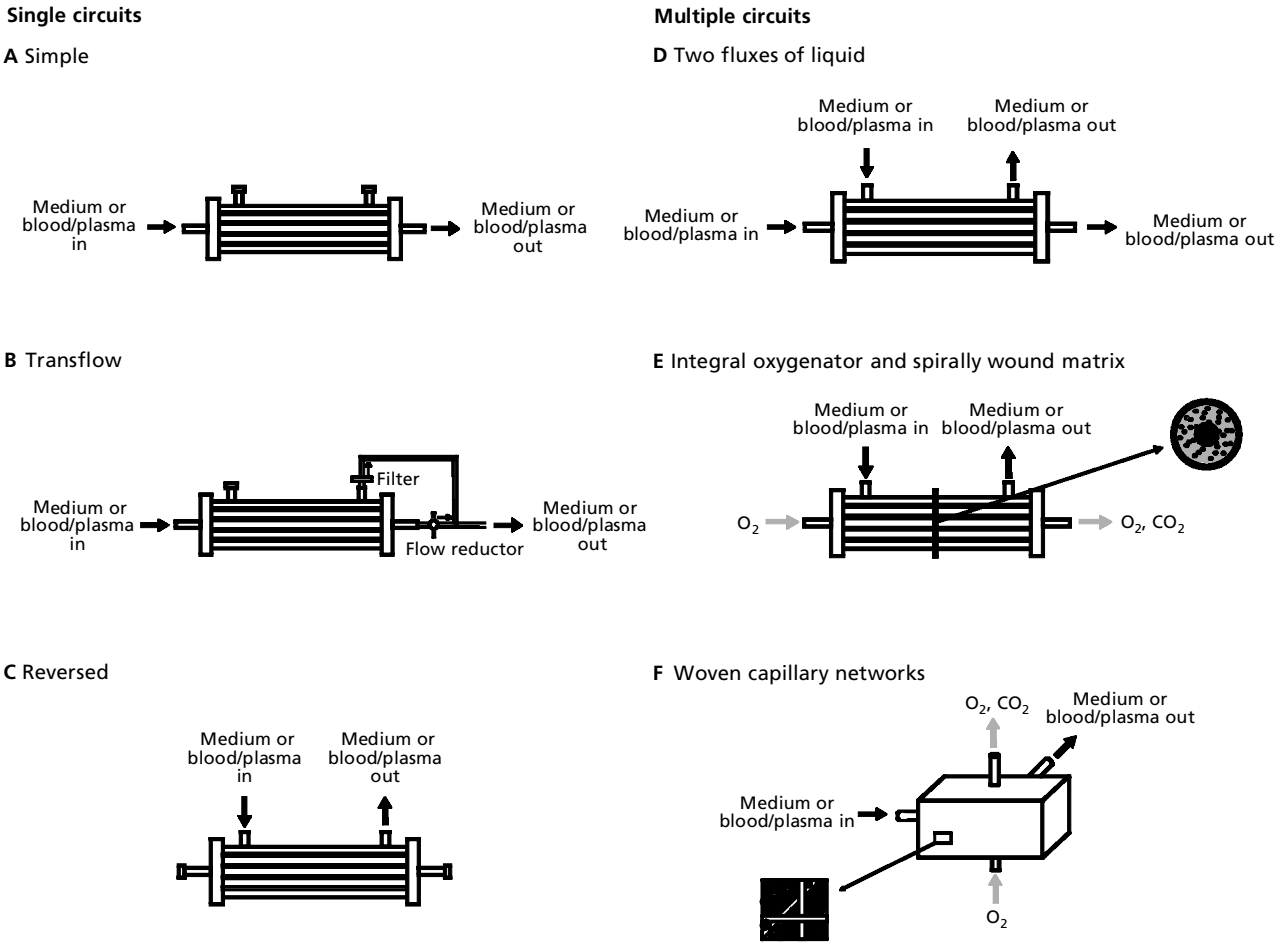

Figure 3 Current designs of hepatocyte HFBs. In single circuits, only the culture medium (or the blood/plasma in the case of BALs) is perfused through the HFB, while two or more fluxes are present in multiple circuits.

the ECS remains relatively constant along the length of the HFB, in contrast to the ICS, where pressure varies. At the inlet, pressure in the ICS is maintained above the ECS pressure, but it decreases along the length of the HFB and can fall below the ECS pressure. Thus, while the initial convective trans-membrane flux is towards the ECS, it can be directed back into the ICS further down the axis (Giorgio et al 1993). Cells near the downstream section of the HFB can therefore be under nutrient-starvation conditions. This problem becomes more severe as the length of the HFB increases (Tharakan et al 1988). In the case of cell suspensions, the phenomenon may cause cells to be pushed and compressed at the downstream end of the HFB (Tharakan 1986).

To avoid a pressure drop along the HFB and thus an axial nutrient gradient, Rozga et al (1993a) imagined a variant of the simple model (Figure 3B). In their transflow system the shell port downstream was open, and the nutrient stream diffused across the HF membrane and exited through the shell-side port. In contrast to the simpler model, the lateral pressure inside the HFs was always greater than the pressure on the shell side, but decreased linearly along the HFB. The flow was therefore always into the shell and solute appearance was faster than in the simpler model (Giorgio et al 1993). A filter was set at the downstream-side port to retain cells in the ECS.

Another single circuit has been described by Iwata et al (1999). Here, the compartments are reversed compared to the simpler model, i.e. cells are cultured in the ICS whereas the medium is perfused through the ECS (Figure 3C). The advantage of this inverted system is the short distance between the cells and the HF membrane. Consequently, even hepatocytes in the central part of the HFs are well fed.

Multiple-circuit systems. Unlike simple circuits, multiple-circuit systems comprise more than one flux. The simplest of such systems comprises an HF cartridge similar to that of single-circuit systems but with an additional liquid flux (Figure 3D). For example, Nyberg et al (1992, 1993b, 1994) used a system with hepatocytes trapped in a collagen gel inside the HF lumen. The medium circulated within the HFs, bringing nutrients to the hepatocytes.

A second flux, consisting of medium (or blood/plasma in a BAL arrangement), flowed in the ECS. In a different multiple system (Patzer II et al 1999; Mazariegos et al 2001, 2002), the cells were located in the ECS and perfused with a slow flux of nutrient solution while a more important flow of medium (or blood/plasma) circulated in the ICS. The advantages of this design are the presence of a support for cell growth and the small diffusion distance for mass transfer of nutrients and waste products.

Flendrig et al (1997) built an HFB that delivered gases and liquid nutrients separately to the cells (Figure 3E). It consisted of a spirally wound non-woven polyester matrix that created a three-dimensional framework for hepatocyte immobilization and aggregation, and of integrated hydrophobic HF membranes for decentralized O_2 supply and CO_2 removal. The medium was perfused through the ECS and was therefore in direct contact with hepatocytes. Gerlach et al (1994a) used an HFB made of three discrete capillary systems: one for plasma inflow, another for oxygenation and CO_2 removal, and the third for plasma outflow (Figure 3F). The advantages of this system are the three-dimensional framework for well-distributed cell perfusion with low metabolite gradients, and well-distributed oxygenation and CO_2 removal. The system has been made even more sophisticated by the addition of a fourth capillary system containing sinusoidal endothelial cells for co-culture (Gerlach et al 1994b). The OXY-HFB recently developed by Jasmund et al (2002) also consisted of two sets of woven capillaries for separate oxygenation and solution perfusion, the hepatocytes being placed in the ECS.

Set-up of hepatocyte HFBs

Types of hepatocyte culture in HFBs

Hepatocytes are adhesion-dependent epithelial cells that require a surface for attachment and proliferation. In-vivo, the cell microenvironment consists mainly of interactions between homotypic or heterotypic cells that affect cellular activity, shape and polarity (Guguen-Guillouzo & Corlu 1993). Recent advances in hepatocyte culture have evidenced the critical influence of the microenvironment on cell activities and clarified some in-vitro conditions that best mimic in-vivo situations. Schematically, three major strategies can be used: (i) favouring cell–cell contact between hepatocytes, (ii) providing hepatocytes with one of the several components that form the hepatic extracellular matrix (a complex mixture of collagens, non-collagenous proteins and carbohydrates) and (iii) use of co-culture to restore the communication of hepatocytes with other liver cell types.

These elements should be taken into account in hepatocyte HFB culture. Thus, a number of techniques are currently used to provide an adhesion support to hepatocytes, including HF surface coating, gel embedding, attachment to microcarriers and co-culture.

Suspensions of freshly isolated hepatocytes. Although hepatocytes are adhesion-dependent cells, suspensions of freshly isolated hepatocytes (Figure 4A) are often used as a quick, simple and reproducible technique in the preparation of HFBs. This approach allows the loading of HFBs with a large number of cells, leading to a high cellular density. Moreover, hepatocytes remain able to metabolize a large number of compounds by functionalization (phase I) and conjugation (phase II) reactions (Guillouzo 1986). Thus, suspensions have the advantage of representing a homogenous hepatocyte population with a cellular density, activity and function comparable to those seen in-vivo. The main disadvantage of suspensions is a modification of hepatocyte membrane architecture caused by the isolation process, since hepatocytes lose their polarity when cultured in suspension, and structural and

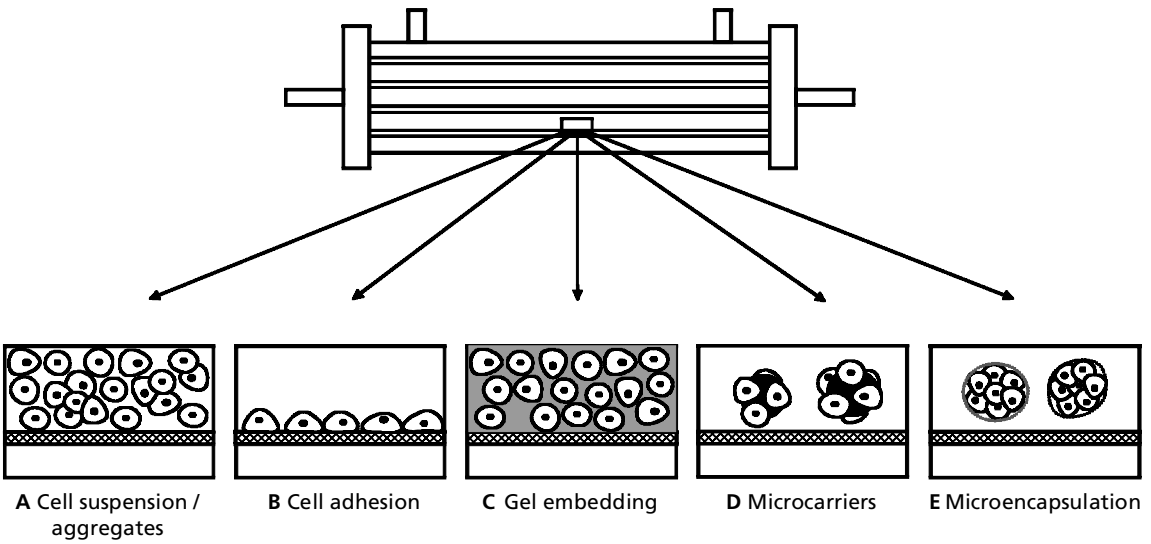

Figure 4 Hepatocyte culture in HFBs. Hepatocytes are simply loaded in the case of suspensions, whereas they can adhere to a support in the case of cell adhesion, gel embedding, microcarriers and microencapsulation.

functional alterations can appear at the level of each hepatocyte (Gebhardt 1986). As a result, hepatocyte viability and functionality in suspension are limited to a few hours after isolation, restricting extended experiments (Gerlach et al 1989). However, it has been reported that hepatocytes seem to spontaneously form aggregates when cultured at high density in suspensions in bioreactors (Gerlach et al 1994a; Flendrig et al 1997; Iwata et al 1999), thus restoring cell–cell interactions and leading to an improved functionality and viability.

Adhesion on artificial membranes. As already discussed, the HF membrane separates cells from the perfusion medium. Its second role is to provide an adhesion surface to hepatocytes (Figure 4B), improving their activity and viability. Hepatocytes are allowed to attach directly to the membrane or a coated surface (Hager et al 1983; Gerlach et al 1990a). Immobilization and growth depend on the nature of the membrane and on the coating components. Such components include soluble basement membrane extracts (Matrigel) (Gerlach et al 1994b), collagen (Vitrogen) (Jauregui et al 1994) and fibronectin (Dixit 1994). A disadvantage of this mode of culture is the low cellular density achievable in HFBs, as all hepatocytes have to be attached to the HF membrane.

Membranes with different chemical and physical properties have been used. Thus, the growth and function of a human hepatoma cell line were compared using six different membranes (Qiang et al 1997): four cellulose-based membranes, namely cellulose acetate, Hemophan (cellulose with $< 1\%$ diethylaminoethoxyl groups), Cuprophan and Bioflux (regenerated celluloses), and two synthetic polymers, polysulfone and SPAN (a sorbitan ester). Polysulfone, SPAN and Hemophan appeared to be ideal membranes for hepatoma cell adhesion, while adhesion to cellulose acetate was limited and that to Cuprophan and Bioflux was nil. Cellular integrity was largely dependent on membrane adhesion, the best results being obtained with polysulfone. The intrinsic structure of membranes contributes markedly to the differential behaviour of hepatocytes on membranes. Although the precise mechanism of cell adhesion has not been elucidated, the importance of an electrically polar surface has been recognized. Hemophan and cellulose acetate are positively charged, polysulfone and SPAN are negatively charged, and the unmodified cellulose membranes Cuprophan and Bioflux are electrically neutral. These results confirm that membranes with hydrophilic groups on their surfaces enhance cell growth and functionality.

Gel embedding. A gel matrix increases the surface area for cell support, yielding a higher cell density and an improved cell functionality. Hepatocytes can be entrapped in gel and cultured in the ECS (Figure 4C) (Takeshita et al 1995; Patzer II et al 1999) or in the ICS (Nyberg et al 1992, 1993b, 1994) of the HFB. Hepatocyte embedding in a collagen I gel at a concentration of about 0.2% has often been used. A disadvantage of this mode of culture is that a steep concentration gradient of nutrients and O_2 may occur throughout the gel layer, resulting in either cell starvation or accumulation of toxic metabolites (Catapano 1996).

Microcarriers. Microcarriers (Figure 4D) are artificial spherical bodies made from a variety of materials including dextran, polystyrene, biologically modified polyhydroxymethylmethacrylate (poly-HEMA) or glass. Compared to membranes, microcarriers provide an increased surface area for hepatocyte adhesion, increasing the cell density achievable in HFBs. Like HF membranes, microcarriers can be coated with different compounds, such as collagen (Arnaout et al 1990; Rozga et al 1993a, 1993b; Chen et al 1996; Sheil et al 1996), fibronectin (Shnyra et al 1990) or chitosan (Yagi et al 1997). The latter is a suitable scaffold for attaching hepatocytes because of its nontoxic and biocompatible nature and its structure, which is similar to that of the glycosaminoglycans found in the liver extracellular matrix (Kawase et al 1997). Biologically modified poly-HEMA microcarriers, and the newly developed multiporous cellulose microcarriers whose pores can host cells, have been reported to be more suitable than dextran microcarriers as a matrix for long-term, high-density hepatocyte cultures (Dixit 1994; Kasai et al 1994). Finally, cryopreserved microcarrier-attached hepatocytes are used to prepare the HepatAssist BAL, since they retain a good viability and functionality after thawing.

Microencapsulation. Hepatocytes entrapped within a gel matrix of calcium alginate (Figure 4E) maintain a good functionality in-vitro (Miura et al 1986; Clément et al 1998; Dixit & Gitnick 1998), but permeability problems can arise for high-molecular-weight substances, such as secreted plasma proteins. In the case of BALs, encapsulation also permits the immunoisolation of hepatocytes. As with microcarriers, hepatocytes retain their functional activity and metabolic capacity after cryopreservation in alginate droplets (Guyomard et al 1996).

Co-cultures. The term co-culture is used when two or more cell types are cultured together. Co-cultures lead to cellular behaviour and physiological responses that do not occur when each cell type is cultured separately. In the case of hepatocytes, co-culture with fibroblasts (Reid & Jefferson 1984), endothelial cells (Morin & Normand 1986) or with another epithelial cell type (Begue et al 1984) have improved their functionality and viability. This technique has been applied, for example, in an HFB made of four capillary systems, one being loaded with liver endothelial cells, and hepatocytes being cultured in the ECS (Gerlach et al 1994b).

Culture and perfusion conditions in HFBs

Perfusion solutions. Optimizing the composition of the perfusion solution can contribute to the long-term maintenance of cell viability and functionality. Most authors use complete media containing amino acids and vitamins in addition to salts. A common medium for hepatocyte culture is thus Williams' E medium (Miyazaki et al 1990), but other media have also been reported (Ehrlich et al 1978;

Rozga et al 1993b; Jauregui et al 1994). Simple balanced salt solutions (BSSs) can also be used, e.g. Dubelcco's phosphate buffer saline or Hanks' BSS. Albumin or serum, growth factors and antibiotics are often included in the perfusion solution. In the case of BALs, the perfusion solution is replaced by the plasma or blood of the patient or animal, although some experiments have also used a culture medium to provide hepatocytes with specific nutrients and to eliminate hepatocyte waste products.

The presence of a carbonate buffer in the solution and 5% CO_2 in the incubator atmosphere ensures a pH around 7.4. If the CO_2 concentration in the gas phase increases, the buffer 4-(2-hydroxyethyl)-1-piperazinyl-ethanesulfonic acid can be added to the medium for greater pH stability. According to human plasma osmolality, osmotic pressure is usually set at 290 mOsm kg^{-1}. Slightly hypotonic solutions are sometimes used to compensate for evaporation during long experiments.

Oxygenation. Special attention must be paid to optimal cell oxygenation, since O_2 plays a key role in hepatocyte adhesion and functions (Flendrig et al 1997). At high cell densities and low O_2 tensions, cells may switch from aerobic metabolism to anaerobic glycolysis (Tharakan et al 1988). Anoxia produces a gradual increase in plasma membrane permeability, initiating cell death. The capacity to supply O_2 to cultures is severely limited by its low solubility in water (0.2 mmol L^{-1} at 37 °C) and by the rapid O_2 consumption of hepatocytes (Tharakan et al 1988). For this reason, O_2 is used as the limiting factor in models of metabolite transport in HFBs (Chresand et al 1987; Brotherton & Chau 1996; Hay et al 2000). A magnetic resonance imaging (MRI) investigation has shown that a diffusion distance not greater than 200–500 μm is necessary to preserve hepatocyte viability in a single-circuit HFB (Macdonald et al 1998).

The first HFB oxygenation system, described by Knazek et al (1972), consisted of silicone tubing through which O_2 and CO_2 diffused readily from the incubator atmosphere to the solution. This oxygenation system has been called indirect oxygenation as it is placed serially in the HFB system, and it is used in all single-circuit HFBs. Direct oxygenation, where the oxygenation system is included directly in the bioreactor (Gerlach et al 1994a; Flendrig et al 1997; Jasmund et al 2002), has also been employed. It was observed that during the first 24 h, hepatocytes in HFBs reduced ammonia concentration in the medium independently of direct or indirect oxygenation. In longer-term cultures, however, the direct method led to enhanced cellular metabolism (Gerlach et al 1990b). The composition of the feeding gas also influences O_2 consumption, an augmented O_2 consumption being observed at higher O_2 tension (Custer & Mullon 1998).

Flow rate. As O_2 is crucial for the maintenance of hepatocyte viability, its diffusion is much more critical than that of other nutrients. High flow rates are thus often used to enhance the amount of dissolved O_2 delivered to hepatocytes, as the rate of solute appearance in the ECS is a function of flow rate (Giorgio et al 1993; Rozga et al 1993a). However, highly elevated flow rates are seldom selected since they increase hepatocyte shear stress and can cause an axial concentration gradient of cellular viability.

Cellular density. The optimal hepatocyte density in HFBs is defined as the maximum that allows each cell to be supplied with sufficient nutrients, including glucose and O_2. Optimal density can be reached stepwise by increasing cell mass as long as cell functionality and viability are maintained. Optimal cell density depends mainly on HFB dimensions and on the mode of cell culture. An equation has been proposed to determine the maximum cell density achievable with a given HFB, based on nutrient consumption, HFB characteristics and number of HFs (Chresand et al 1987). For cell suspensions, a tightly packed HF bundle will support a high local density in the interfibre space, but the available volume is limited. Conversely, generous HF spacing allows a large volume for cells but a lower local density.

Primary hepatocytes behave differently depending on their density in HFBs (Gerlach et al 1994a). When hepatocytes were seeded at low density between the HFs, they adhered to the membrane surface and flattened, whereas at high densities they additionally formed aggregates in the ECS.

Validation of hepatocyte HFBs

The validation of an HFB is an important step that is achieved by confirming cell viability and functionality, since the performances of HFBs directly depend on these factors. Most cellular viability tests rely on direct cell coloration (using a dye such as trypan blue or fluorescein), but this test cannot monitor cell viability during experiments since HFBs are closed systems that prevent cell sampling. Consequently, other tests must be used to quantify hepatocyte viability during experiments, e.g. by monitoring the release of intracellular enzymes or metabolic products.

For the validation of HFBs, we distinguish here between monitoring of O_2 consumption and levels of endogenous biomolecules (e.g. enzymes and proteins) on the one hand, and the capacity to metabolize drugs or endobiotics on the other.

Monitoring of O_2 consumption and levels of endogenous biomolecules

O_2 consumption is currently used to assess cellular viability as it reflects the biochemical state of the cellular population. Insulin consumption can be used as an indirect measure of hepatocyte growth since insulin stimulates hepatocyte proliferation. The concentration of liver-specific proteins such as albumin, fibrinogen, haptoglobin, creatinine and transferrin reflects the synthetic capacity of hepatocytes associated with the preservation of a transcriptional activity.

The specific release of intracellular enzymes (lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamic pyruvic transaminase (GPT), glutamate dehydrogenase (GLDH), γ-glutamyl transpeptidase (GGT) and transaminases) caused by membrane leakage is a criterion of cell integrity. Finally, some amino acids are sometimes monitored because they are suspected of playing an unknown role in hepatic encephalopathy, a syndrome seen in patients with liver diseases characterized by personality change, intellectual impairment and a depressed level of consciousness (Sheil et al 1996).

Metabolic studies of endobiotics and drugs

Overview. In its broadest sense, metabolism includes all the chemical reactions occurring in the body, and principally the catabolism and anabolism of endogenous compounds and the breakdown of nutrients to produce energy (Marieb 1993). Metabolism also includes the derivatization of endobiotics and drugs to inactivate them and increase their hydrophilicity to facilitate excretion. Such reactions of derivatization are divided into functionalization and conjugation. Functionalization (phase I) reactions involve the creation or modification of functional groups in a substrate molecule by oxidation, reduction or hydrolysis, whereas conjugation (phase II) reactions involve the coupling of an endogenous molecule with the substrate, yielding a conjugate (Testa 1995). Table 1 presents various endobiotics and drugs whose metabolism was used to validate hepatocyte HFBs, or was investigated using this methodology.

Metabolic studies of endobiotics. Ammonia elimination is a relevant metabolic reaction for testing the performance of BALs as ammonia is one of the neurotoxic species related to hepatic encephalopathy (De Bartolo et al 1999). Ammonia is detoxified during ureagenesis, reflecting the presence of enzymes of the urea cycle. For in-vitro tests, ammonia elimination and urea synthesis are evaluated after the administration of NH_4Cl. In humans and animals coupled to a BAL, ammonia is naturally present in the blood as a result of protein degradation.

Bilirubin uptake, conjugation and excretion is a complex hepatic function (Wolf & Munkelt 1975). Free bilirubin is conjugated to a monoglucuronide and a diglucuronide by the hepatic enzyme uridine diphosphate-glucuronyltransferase, rendering it water-soluble and excretable into the bile. It is thus considered as an endogenous marker of conjugation reactions. Other markers include cytidine, whose deamination to uridine by the enzyme cytidine deaminase reflects the functionality of cytosine arabinoside metabolism (Hager et al 1983). Like O_2 consumption, glucose depletion in the medium is an indicator of the biochemical state of hepatocytes. Finally, galactose is eliminated by the liver after uptake into hepatocytes by a carrier-mediated transport process and conversion to glucose by enzymes including galactokinase and uridyltransferase. The capacity to eliminate galactose is also used in clinical diagnosis as an indicator of functional liver cell mass.

Metabolic studies of drugs. Diazepam and lidocaine are sometimes used to evaluate the metabolic function of hepatocytes. Their total metabolic activity reflects the status of the cytochrome P450 pathway, indicating that hepatocytes have the capacity to oxidize and/or reduce drugs. Diazepam and lidocaine are thus considered as phase I markers. The metabolic scheme of diazepam is represented in Figure 5.

The glucuroconjugation of 19-nortestosterone and 4-methylumbelliferone is used as evidence of conjugation (phase II) reactions. The same is true of phenolsulfonphthalein, whose glucuronidation can easily be monitored by UV spectrometry.

Applications of hepatocyte HFBs

A physiologically relevant environment allows hepatocyte HFBs to be used for a number of purposes. Three main types of application can be distinguished: (i) synthesis of cellular products, (ii) use as bioartificial livers (BALs) and (iii) use in metabolism and transport studies.

Synthesis of cellular products using hepatocyte HFBs

In many cases, tumour-derived cell lines are used in hepatocyte HFBs when the objective is the synthesis of cellular products. The advantages of cell lines over primary cell cultures are a continuous secretion, an easier purification and a better yield of cellular products. Moreover, cell lines are immortal (which facilitates cloning), their culture is easier, and they reach a higher cellular density.

Thus, purified hepatitis B surface antigen can be produced from Alexander hepatoma cells grown in HFBs (McAleer et al 1983). At the time of these studies, human hepatitis B vaccines contained hepatitis B surface antigen obtained from the plasma of humans infected with the hepatitis B virus. Given the dangers associated with the use of human blood, however, a promising alternative source of antigen for vaccine production was afforded by the Alexander hepatoma cell line, which carries the genome of hepatitis B virus and secretes its surface antigen.

HepG2 cells, another human hepatoma cell line, can be cultured in HFBs to produce the HepG2 crude conditioned medium (CCM), which contains high levels of proteins, including the endothelial cell growth factor and platelet-derived growth factor (Liu et al 1991). HepG2 CCM was found to be comparable to or even better than fetal calf serum in supporting the growth of cells in-vitro. Serum is traditionally added to cell cultures to provide nutrients, notably growth factors. However, as the sources of serum are limited and its composition may vary, the development of serum substitutes is a priority. HepG2 CCM appears to be a good candidate.

Hepatocyte HFBs as BALs

Currently, the major application of hepatocyte HFBs is as BALs to sustain patients suffering from ALF when a donor liver is not available (i.e. as a bridge to transplantation). ALF is associated with a very high mortality rate, and its symptoms include encephalopathy, reduced

Table 1 A selection of metabolic reactions investigated using hepatocyte HFBs.

	Reactions	Markers	Analytical method	References
Endobiotics				
Ammonia	Urea formation	Ammonia	HPLC/UV	Nyberg et al (1993c, 1993b)
		Urea	UV	Flendrig et al (1997); Hager et al (1983); Sielaff et al (1997)
			HPLC/UV	Nyberg et al (1993b)
Bilirubin	Glucuronidation	Mono- and di-glucuronide	HPLC/UV	Arnaout et al (1990)
Cytidine	Deamination	[^{14}C]Uridine	Radioactivity	Hager et al (1983)
Galactose	Conversion to glucose	Galactose	UV	Ellis et al (1996); Flendrig et al (1997); Gerlach et al (1994a)
Glucose	Glycolysis and gluconeogenesis	Glucose	Test strips	Flendrig et al (1997)
Drugs				
Diazepam	Oxidation	Diazepam	GLC	Hager et al (1983)
			HPLC/UV	Custer & Mullon (1998); Jasmund et al (2002); Jauregui et al (1994)
	Hydroxylation	Temazepam		
	N-Demethylation	Nordiazepam		
	Hydroxylation and N-demethylation	Oxazepam		
Lidocaine	Oxidation	Lidocaine	GC	Yagi et al (1997)
			HPLC/UV	Flendrig et al (1997); Iwata et al (1999); Nyberg et al (1993b)
			Immunoassays	Gerlach et al (1994b); Shatford et al (1992)
			Fluorescence polarization	Jauregui et al (1995)
	N-Dealkylation	Monoethylglycinexylidide (MEGX)	HPLC/UV	Flendrig et al (1997); Iwata et al (1999); Nyberg et al (1993b)
			Immunoassays	Gerlach et al (1994b); Shatford et al (1992); Sussman et al (1994)
			Fluorescence polarization	Jauregui et al (1995)
	N-Dealkylation	Glycinexylidide	HPLC/UV	Flendrig et al (1997); Nyberg et al (1993b)
	Amide hydrolysis	Xylidine	HPLC/UV	Flendrig et al (1997)
	Hydroxylation	3-OH-Lidocaine	HPLC/UV	Nyberg et al (1993b); Sussman et al (1994)
	N-Dealkylation and hydroxylation	3-OH-MEGX	HPLC/UV	Nyberg et al (1993b)
Midazolam	Oxidation	Midazolam	Immunoassays	Gerlach et al (1994b)
Cyclosporine	Hydroxylation	First-generation monohydroxylation products	HPLC/UV	Rozga et al (1993a)
	N-Demethylation	First-generation products of N-demethylation	HPLC/UV	
19-Nortestosterone	Glucuronidation	19-Nortestosterone glucuronide	GC/MS	
	Isomerisation	19-Norepicholanolone and 19-norepiandrosterone	GC/MS	
Phenol-sulfonphthalein (PSP)	Glucuronidation	PSP-glucuronide	UV	Jauregui et al (1994)
4-Methyl-umbelliferone (4MU)	Conjugation	4MU	HPLC/UV	Nyberg et al (1993b)
	Glucuronidation	4MU-glucuronide		
	Sulfation	4MU-sulfate		

Additional details available from Corinne Planchamp (Corrine.Planchamp@hcuge.ch).

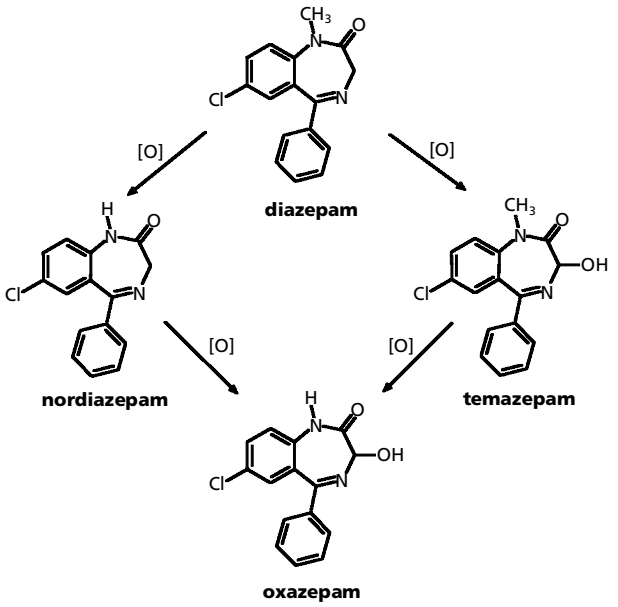

Figure 5 Cytochrome P450-catalyzed oxidation of diazepam to temazepam, nordiazepam and oxazepam.

albumin synthesis, prolonged prothrombin time and elevated bilirubin and ALT levels (Sussman et al 1992). Orthotopic liver transplantation is currently the treatment of choice for patients with ALF, but many of them cannot survive until a donor organ is available. Thus, the use of BALs increases the chances of survival of patients suffering from ALF, providing them with temporary liver assistance until transplantation.

Previous methods to support patients suffering from ALF were based primarily on extracorporeal blood purification, using physical or biological techniques. Physical techniques include dialysis, affinity chromatography, charcoal adsorption and ion exchange, whereas biological techniques include extracorporeal perfusion through animal livers, cross-circulation with animals or cadavers, as well as simple exchange transfusion or plasmapheresis (Allen et al 2001). However, none of these methods was satisfactory enough to gain wide clinical application. The use of bioreactors loaded with hepatic cells serves as an intermediate between physical and biological techniques. Beside HFBs, several other BAL designs have been proposed, including direct hemoperfusion of microencapsulated hepatocytes in an extracorporeal chamber (Dixit & Gitnick 1998) and plate dialyzers (Cuervas-Mons et al 2001). However, the BALs that have currently reached clinical evaluation are all based on HFBs (Patzer II 2001).

BALs are expected to replace many liver functions, including metabolism and secretion. The information reported in the previous sections finds application in the development of BALs and in obtaining an optimal functionality of hepatocytes in HFBs. The development of BALs involves in-vitro investigations (Table 2) and in-vivo animal experiments (Table 3) before being cleared for validation in humans (Table 4). Tables 2–4 list various characteristics of HFBs, and how their viability and functionality can be checked during use. In addition to these tests, a number of biological parameters can be monitored in patients during clinical trials, including vital signs, neurological monitoring, whole blood cell counts and coagulation tests. Our aim here is not to provide an exhaustive list of all the HFBs used as BALs, but to give an overview of the main systems.

In an HFB used as a BAL (Figure 6), the patient's blood or plasma comes into contact with liver cells of human or animal origin. Perfusing plasma instead of whole blood eliminates the problems caused by hemolysis, thrombocytopenia, clot formation, embolization and the need to use heparine (Demetriou et al 1995), but whole blood has the advantage that erythrocytes improve O_2 delivery to hepatocytes. A charcoal column is often included in the perfusion system upstream from the HFB to adsorb part of the toxins present in plasma (Sheil et al 1996; Watanabe et al 1997). Different kinds of liver cells can be loaded in HFBs. Primary human hepatocytes are the optimal choice, but they are in short supply. Transformed human liver cells are an alternative as they grow to high cellular density. However, they are inferior to primary cells in accomplishing hepatic functions and bear the potential risk of tumour transmigration from the BAL into the patient's circulation (Nyberg et al 1993a, 1994). Primary animal hepatocytes, on the other hand, have a limited life span in-vitro and have the disadvantage of an increased risk of immunological complications and potential xenozoonosis. For clinical use, porcine hepatocytes are preferred to rodent hepatocytes since they have higher cell yields.

The immunological isolation of cells is important to reduce complications. However, it is difficult to determine the optimal molecular weight cut-off of HF membranes suitable for BALs. Indeed, a lower cut-off implies a smaller risk of immune reactions on both sides of the membrane, but also a less efficient removal of protein-bound toxins. For example, HF membranes with a cut-off of 100 kDa act as impermeable barriers to immunoglobulins ($\sim$150 kDa) and complement ($>$200 kDa), and prevent the transmission of pathogens, while allowing soluble and albumin-bound toxins (albumin $\sim$ 60 kDa) to permeate. In this perspective, the porcine hepatocyte BAL HepatAssist has an unexpectedly large HF porosity of 0.2 μm (0.1 μm $\sim$ 1000 kDa). The argument here is that the use of plasma perfusion (no direct contact between immune cells in the blood and BAL cells), purified isolated hepatocytes (no endothelial cells and other highly antigenic cells) and HFs with a large porosity represents a good compromise between an efficient hepatic function and the prevention of immune reactions (Demetriou et al 1995).

Five HFB bioartificial livers (all of which have the cells in the ECS) have recently been tested clinically (Table 4). Whereas the Hepatix ELAD device uses a human hepatoblastoma cell line (C3A cells derived from the HepG2 cell line), the other four use primary porcine hepatocytes. The HepaAssist BAL is currently undergoing Phase II/III randomized, multicentre clinical safety/efficacy evaluation.

Table 2 A selection of in-vitro tests conducted with hollow-fibre BALs.

Hollow-fibre cartridge model/bioreactor design/ manufacturer	Cell type	Markers of hepatocyte function		References
		Drug metabolism	Endobiotics (metabolism, consumption, synthesis, release)	
Vitafiber/single circuit (Figure 3A)/ Amicon, USA Dowex cell culture tube c/HFBI-05/single circuit (Figure 3A)/ BioRad Lab, USA	Rat hepatoma cell line H4-II-E	NS	Bilirubin metabolism, glucose consumption, LDH, AST and GTP release	Wolf & Munkelt (1975)
Vitafiber XM50/single circuit (Figure 3A)/Amicon, USA	Neonatal murine hepatocytes	Diazepam	Glucose consumption, cytidine metabolism, albumin, fibrinogen, transferrin and urea synthesis	Hager et al (1983)
H1P100/multiple circuits (Figure 3D)/Amicon, USA	Primary rat hepatocytes	Lidocaine	Albumin synthesis, O_2 consumption	Shatford et al (1991, 1992)
Minikap 225/single circuit (Figure 3B)/Microgon, USA	Primary rat hepatocytes	Cyclosporine, 19-nortestosterone	NS	Rozga et al (1993a)
H1P100/multiple circuits (Figure 3D)/Amicon, USA	Primary rat hepatocytes or HepG2 cells	Lidocaine, 4-methylumbelliferone	Albumin and urea synthesis, lactate, O_2, glucose and insulin consumption, aa metabolism	Nyberg et al (1992, 1994)
NS/single circuit (Figure 3A)/ Grace & Co, USA	Primary rat hepatocytes	Diazepam, phenolsulfonphtalein	NS	Jauregui et al (1994)
Home-made/multiple circuits (Figure 3F)/NS	Primary pig hepatocytes	Lidocaine, midazolam	Galactose metabolism, LDH, AST, GPT, GLDH and GGT release, albumin synthesis	Gerlach et al (1994b)
Culture Flo G/single circuit (Figure 3A)/Asahi Medical, Japan	Primary rat hepatocytes	NS	Urea and albumin synthesis, ammonia metabolism, AST release	Takeshita et al (1995)

Table 2 (*Cont.*)

Hollow-fibre cartridge model/bioreactor design/ manufacturer	Cell type	Markers of hepatocyte function		References
		Drug metabolism	Endobiotics (metabolism, consumption, synthesis, release)	
Cellmax/single circuit (Figure 3A)/ Cellco, USA	Primary rat hepatocytes	Lidocaine	LDH release, urea synthesis	Yagi et al (1997)
Home-made/multiple circuits (Figure 3E)/NS	Primary pig hepatocytes	Lidocaine	Galactose and aa metabolism, urea and protein synthesis, LDH, AST and GPT release, glucose synthesis	Flendrig et al (1997)
NS/multiple circuits (Figure 3D)/Althin Medical, USA	Primary porcine hepatocytes	NS	Urea and albumin synthesis, O_2 consumption	Sielaff et al (1997)
HepatAssist BAL2000/single circuit (Figure 3A)/Circe Biomedical, USA	Primary porcine hepatocytes	Diazepam	O_2 consumption	Custer & Mullon (1998)
Duo-Flux M170D/single circuit (Figure 3C)/JMS, Japan	Primary porcine hepatocytes	Lidocaine	Urea synthesis, galactose metabolism	Iwata et al (1999)
OXY-HFB (modified Quadrox)/ multiple circuits (Figure 3F)/ Jostra, Hechingen, Germany	Primary pig hepatocytes	Diazepam	Urea, lactate and albumin synthesis, O_2 and glucose consumption	Jasmund et al (2002)

NS, non specified; aa, amino acids; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GPT, glutamic pyruvic transaminase; GLDH, glutamate dehydrogenase; GGT, γ-glutamyl transpeptidase. Additional details available from Corinne Planchamp (Corrine.Planchamp@hcuge.ch).

Table 3 A selection of in-vivo animal tests conducted with hollow-fibre BALs.

Hollow-fibre cartridge model/bioreactor design/ manufacturer	Animal species	Cell type	Markers of hepatocyte function		References
			Drug metabolism	Endobiotics (metabolism, consumption, synthesis, release)	
Vitafiber/single circuit (Figure 3A)/ Amicon, USA	Rat	Rat hepatoma cell line H4-II-E	NS	Bilirubin metabolism	Wolf & Munkelt (1975)
Dowex cell culture tube c/HFBI-05/single circuit (Figure 3A)/ BioRad Lab, USA	Rat	Primary rat hepatocytes	NS	Bilirubin metabolism	Arnaout et al (1990)
Minipure ES 1-12/single circuit (Figure 3A)/ Microgon, USA	Dog	C3A cells	NS	Albumin synthesis, ALT release	Sussman et al (1992)
Cell-pharm 240-322/single circuit (Figure 3A)/ UniSyn Fibertec, USA	Dog	Primary porcine and dog hepatocytes	NS	Glucose consumption, lactate synthesis, LDH and AST release	Rozga et al (1993a)
Minikros M22M-030-01N/single circuit (Figure 3B)/ Microgon, USA	Dog	Primary porcine hepatocytes	NS	Glucose consumption, lactate synthesis, LDH and AST release, ammonia metabolism	Rozga et al (1993b)
Z22M-060-01X (HepatAssist)/ single circuit (Figure 3A)/ Microgon, USA	Rabbit	Primary rat hepatocytes	Lidocaine, 4-methylumbelliferone	Albumin and glucose synthesis, aa and ammonia metabolism, O_2 consumption	Nyberg et al (1993b)
H1P100/multiple circuits (Figure 3D)/Amicon, USA	Rabbit	Primary rabbit hepatocytes	Lidocaine, diazepam	Bilirubin and ammonia metabolism, ALT and LDH release	Jauregui et al (1995)
NS/single circuit (Figure 3A)/NS	Pig	Primary porcine hepatocytes	NS	Lactate, aa and ammonia metabolism, albumin synthesis	Sheil et al (1996)
Z22M-060-01X (HepatAssist)/ single circuit (Figure 3A)/ Microgon, USA	Pig	Primary porcine hepatocytes	NS	NS	Gerlach (1997)
Home-made/multiple circuits (Figure 3F)/NS	Dog	Primary porcine hepatocytes	NS	ALT and AST release, ammonia metabolism	Patzer II et al (1999)
Excorp Medical BLSS/multiple circuit (Figure 3D)/NS	Dog	Primary porcine hepatocytes	NS	ALT and AST release, ammonia and bilirubin metabolism	Chen et al (2001)
TECA BLSS/single circuit (Figure 3A)/Hong Kong Co					

NS, non specified; aa, amino acids; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GPT, glutamic pyruvic transaminase; GLDH, glutamate dehydrogenase; GGT, γ-glutamyl transpeptidase. Additional details available from Corinne Planchamp (Corrine.Planchamp@hcuge.ch).

Table 4 A selection of clinical studies of hollow-fibre BALs.

Hollow-fibre cartridge model/ bioreactor design/manufacturer	Cell type	Markers of hepatocyte function		Clinical protocol	Number of patients	References
		Drug metabolism	Endobiotics (metabolism, consumption, synthesis, release)			
HepatAssist (Z22M-060-01X)/ single circuit (Figure 3A)/ Microgon, USA	Primary porcine hepatocytes	NS	AST, ALT and LDH release, ammonia and bilirubin metabolism, glucose consumption, lactate synthesis	One to three treatments lasting 6 h	*c*. 120	Rozga (1993b, 1994); Demetriou et al (1995); Chen et al (1996); Watanabe et al (1997); Mullon & Pitkin (1999); Samuel et al (2002)
Hepatix ELAD/single circuit (Figure 3B)/NS	Human liver cell line (C3A)	Lidocaine, caffeine	Galactose, ammonia, and bilirubin metabolism, albumin synthesis, O_2 consumption	Uninterrupted treatment for an average of 62 h	*c*. 30	Kelly & Sussman (1994); Sussman et al (1994); Ellis et al (1996)
Home-made/multiple circuits (Figure 3F)/NS	Primary porcine hepatocytes	NS	NS	Uninterrupted treatment for 40 h	1	Gerlach (1997)
Excorp Medical BLSS/multiple circuit (Figure 3D)/NS	Primary porcine hepatocytes	NS	AST and ALT release, ammonia, bilirubin and lactate metabolism, albumin synthesis	One or two treatments lasting 12 h	4	Mazariegos et al (2001, 2002)
TECA BLSS/single circuit (Figure 3A)/Hong Kong Co Ltd	Primary porcine hepatocytes	NS	ALT and AST release, ammonia and bilirubin metabolism	One treatment of 4–5 h	6	Xue et al (2001)

NS, non specified; aa, amino acids; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GPT, glutamic pyruvic transaminase; GLDH, glutamate dehydrogenase; GGT, γ-glutamyl transpeptidase. Additional details available from Corinne Planchamp (Corrine.Planchamp@hcuge.ch).

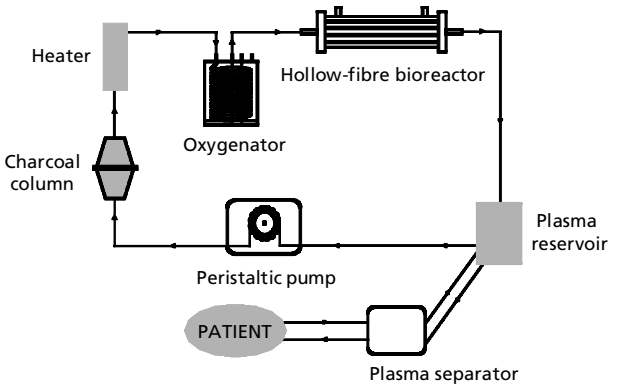

Figure 6 Schematic illustration of a hollow-fibre BAL. Toxic compounds in the blood of patients are partially adsorbed on activated charcoal before being metabolized by the primary hepatocytes or liver-derived cells contained in the HFB. The plasma separator is absent when whole blood is perfused through the system.

The other four BALs are at various stages of Phase I/II clinical safety/efficacy evaluation.

Available clinical reports indicate that treatment with BALs was well tolerated by all patients with evidence of clinical improvement, and that patients were transplanted successfully after BAL treatment (6–7 h). This demonstrates that BALs can indeed be used as a bridge to transplantation for patients with ALF. A few cases of spontaneous recovery without transplantation were even reported (Kelly & Sussman 1994; Samuel et al 2002). In spite of these encouraging preliminary reports, no BAL device has yet received FDA approval for use in ALF.

Hepatocyte HFBs as a tool to study drug metabolism and transport

There are two main and quite distinct reasons for which drug metabolism experiments are carried out using hepatocyte HFBs. The first reason is to test the functionality and viability of the hepatocytes contained in the HFB, as discussed above. The second is to use HFBs as cellular models to study drug metabolism and transport. Liver cell lines, which are dedifferentiated and often lack important metabolic activities, should not be used. For instance, the cytochrome P450 and conjugation activities of HepG2 cells are respectively 200 and 30 times lower than those of primary rat hepatocytes (Nyberg et al 1994). Such cells can nevertheless be used in BALs since high yields compensate for low activities.

Table 5 summarizes the main advantages and disadvantages of HFBs in the study of drug metabolism and transport. HFBs have several advantages over the conventional plate or suspension cultures. In particular, HFBs mimic the in-vivo environment more closely, and hepatocytes are continuously provided with nutrients and O_2, allowing extended experiments. HFBs facilitate sampling, as perfusion solutions are already separated from cells by the semi-permeable membrane of the HFs. Moreover, the dynamic aspect of the system allows continuous measurements of perfusate and changes in perfusion conditions, making it possible to assess the influence of several factors during a single experiment. Although HFBs have many apparent advantages when studying drug metabolism and transport, they are seldom used for this purpose, probably due to their cost and difficult set-up compared to suspensions and plate cultures.

In our laboratory, an HFB containing freshly isolated rat hepatocytes in suspension was used to examine the metabolic chiral inversion of (*R*)-ibuprofen (Figure 7) and the transport mechanism of MRI contrast agents (Figure 8). The use of a hepatocyte HFB to study the chiral inversion of (*R*)-ibuprofen allowed us to prolong the duration of experiments to up to 10 h, compared to 4 h in hepatocyte suspensions (Freshney 2000). This prolonged duration of experiments is due mainly to hepatocytes being better supplied with nutrients and O_2, and it allows the study of slow metabolic reactions.

Hepatocyte HFBs also offer a very interesting tool for the study of the transport mechanisms of contrast agents by MRI, as illustrated by a recent study (Planchamp et al submitted). First, the plastic composition of the HFBs is compatible with the magnetic field. Second, their high cellular density allows the uptake of contrast agents to be detectable by MRI. Finally, the dynamic aspect of the system ensures a continuous perfusion of the hepatocytes inside the magnet with a 37 °C oxygenated solution. Several solutions can be perfused through the HFB in the course of a single experiment. Thus, the uptake and excretion mechanisms of several contrast agents could be investigated successively using the same HFB, thus reducing the number of experiments and animals. Uptake was monitored during perfusion with a solution containing the contrast agent, while excretion was studied during a subsequent perfusion with a solution free of contrast agent (Figure 8).

Table 5 Advantages and limitations of HFBs in the study of drug metabolism and transport.

Advantages	Limitations
Long duration of experiments	Complicated set-up
Study of slow metabolism	High costs
High cell density achievable, easy sampling	Inhomogeneous distribution of nutrients and cells
Continuous measurements of perfusate	
Simultaneous monitoring of several factors	

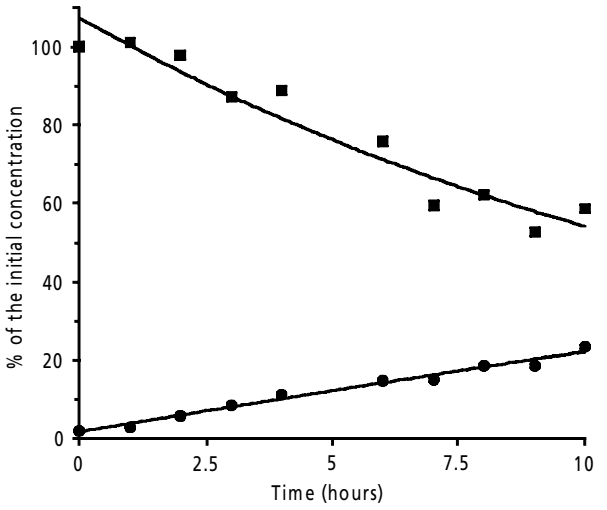

Figure 7 Incubation of *(R)*-ibuprofen in an HFB (Minikros Sampler M22M 030 01N, Spectrum, Rancho Dominguez, USA) containing 4×10^7 hepatocytes per mL. The modified Hanks' solution (MHS) containing the substrate (48.4 μM) was perfused through the HFB at a flow rate of 75 mL min^{-1}. Hepatocyte viability was checked regularly by dosing the release of AST and LDH in the perfusate. ■, *(R)*-ibuprofen; ●, (*S*)-ibuprofen.

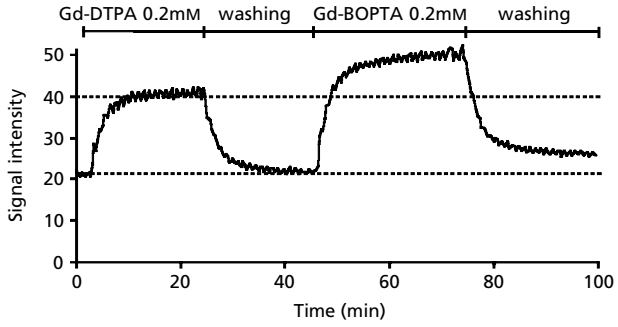

Figure 8 Representative signal intensity over time of a cross-section of an HFB (Minikros Sampler M15E 260 01N, Spectrum, Rancho Dominguez, USA) containing 4×10^7 hepatocytes per mL during the perfusion of an extracellular (Gd-DTPA, Gadopentetate dimeglumine, Magnevist) and an intracellular contrast agent (Gd-BOPTA, Gadobenate dimeglumine; MultiHance). The HFB was perfused with MHS, MHS containing 0.2 mM Gadopentetate dimeglumine (20 min), MHS (20 min), MHS containing 0.2 mM Gadobenate dimeglumine (30 min), and finally MHS (30 min) at a flow rate of 100 mL min^{-1}. Hepatocyte viability was checked regularly by measuring O_2 consumption and the release of AST and LDH in the perfusate. Dashed lines indicate the baseline and the maximal signal intensity obtained with the extracellular contrast agent Gadopentetate dimeglumine, which clearly behaves differently from the intracellular contrast agent Gadobenate dimeglumine.

Conclusion

HFBs have a number of advantages compared to traditional hepatocyte cultures. Their tri-dimensional matrix provides cells with an environment close to the physiological one. The perfusion of a solution supplies hepatocytes continuously with nutrients and O_2. The semi-permeable membrane of the HFs affords a physical separation of solution and cells, protects the latter from a high shear stress and ensures a stable environment. Finally, the large surface of the HFs allows an efficient transfer of O_2, a critical factor for the survival of hepatocytes. Taken together, these aspects ensure the long-term viability and functionality of hepatocytes in culture.

Depending on experiments, various designs of HFB and hepatocyte culture conditions can be chosen. Although the simple HFB design first described (Knazek et al 1972) is still in use, more complicated systems have been developed to improve the distribution of cells and nutrients. Long-term cultures can be obtained when hepatocytes are attached to a support such as HF membranes, microcarriers or gel embedding, while suspensions of hepatocytes present the advantages of being simpler and yielding a higher cellular density. When setting up an HFB system, special attention must be paid to efficient hepatocyte oxygenation since O_2 plays a key role in hepatocyte viability and function. A number of parameters should be monitored to validate the functionality of hepatocyte HFBs, e.g. nutrient consumption, production of endogenous compounds and the capacity to metabolize drugs.

HFBs containing immortalized cells derived from hepatocytes have been used for the synthesis of cellular products such as the surface antigen of hepatitis B for vaccine production, or growth factors for the substitution of serum in cell culture solutions. Currently, however, hepatocyte HFBs are mainly used as BALs for patients suffering from ALF and awaiting liver transplantation, several hollow-fibre BALs now being under clinical evaluation (Sussman et al 1994; Demetriou et al 1995; Chen et al 1996; Watanabe et al 1997). Finally, hepatocyte HFBs are an interesting tool for the investigation of drug metabolism and transport. They make long-lasting experiments possible, allowing slow metabolic reactions to be investigated. Moreover, the dynamic set-up of HFBs allows continuous monitoring of metabolites and in-process modification of experimental conditions. Despite these advantages, however, the high cost and difficult set-up of hepatocyte HFBs probably explain their limited use in drug metabolism and transport studies.

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