Towards application of a human liver cell line for use in the AMC bioartificial liver

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Liver support therapy: 
an overview of the AMC bioartificial liver research (1995-2004)

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Abstract

Acute liver failure (ALF) is a disease with a mortality of 60–90% depending on the cause. Only high-urgency liver transplantation is able to increase survival compared to standard intensive care therapy. Liver transplantation is hampered by the increasing shortage of organ donors, resulting in a high incidence of patients with ALF dying on the transplantation waiting list. Amongst a variety of liver assist therapies, bioartificial liver (BAL) therapy is marked as the most promising solution to bridge ALF patients to liver transplantation or to liver regeneration, since several BAL systems showed significant improvement of survival time in experimental animals with irreversible ALF. One of these systems has been developed at the Academic Medical Center in Amsterdam, The Netherlands – the AMC-BAL. This overview describes the development of the AMC-BAL based on porcine hepatocytes which was started 10 years ago. Positive results of *in vitro* functionality and *in vivo* safety and efficacy led to a successful phase I study in 12 ALF patients in Italy. However, xenotransplantation legislation in many European countries prohibits the use of porcine hepatocytes in clinically applied BAL systems. The future of the BAL, therefore, resides in the development of a human-derived hepatocyte cell line as biocomponent of BAL systems.
Introduction

Mortality of acute liver failure (ALF) remains high despite maximal supportive intensive care treatment. Mortality ranges from 60 to 90% depending on the cause of underlying liver disease. Since the 1950s many supportive modalities have been added to standard therapy of the acute failing liver. These additional therapies range from drug treatment to isolated liver perfusion, liver support devices and ultimately, liver transplantation. At present, orthotopic liver transplantation (OLT) is the only effective treatment of ALF improving survival in comparison to standard intensive care treatment. Emergency OLT is associated with a 1-year survival of 60–90%, depending on the cause of ALF and the selection criteria applied for OLT.1-7 However, due to the shortage of donor livers, a considerable number of patients with ALF die while on the waiting list for OLT. Despite the efforts to increase the donor liver pool by using split livers, living related donor livers and marginal livers, the availability of donor livers is far less than the demand. Because of these high mortality rates and the increasing waiting times for transplantation over the last years1, interest has been renewed in techniques for providing temporary liver support to bridge the liver failure patient to OLT or liver regeneration. These techniques can be grossly divided into non-biological and biological liver support. Many attempts have been made to develop non-biological liver support therapies based on detoxification of the patient’s blood8-12, but all revealed limited or no success.13 The success of OLT indicates the importance of not only restoration of failing detoxification but also restoration of metabolic functions in patients with ALF. Since these functions can best be carried out by hepatocytes, more is to be expected from biological liver support systems.

Besides ALF, other indications for BAL treatment are acute on chronic liver failure and liver support therapy in post-hepatectomy liver failure. Furthermore, a BAL is potentially capable of improving a patient’s condition and converting patients from a non-transplantable to a transplantable state.

Bioartificial liver (BAL) systems are extracorporeal systems that rely on the functionality of hepatocytes from xenogeneic or human origin. Although an increasing number of BAL devices have been developed, only eleven different BAL systems have ultimately passed the test of clinical application.13 The AMC-BAL is one of these systems.

Development of the AMC-BAL

In the early 1990s, Flendrig et al.14 developed a novel BAL system based on a bioreactor with an integral oxygenator and a spirally wound, non-woven polyester matrix for small aggregate hepatocyte three-dimensional culture, which outperforms monolayer cultures.
The advantages of the AMC-BAL above other systems are:

1. Low substrate and metabolite gradients in a high-density hepatocyte culture.
2. Direct contact (without interference of a membrane) between the culture medium of plasma and the hepatocytes resulting in optimal bidirectional mass transfer.
3. Decentralized oxygenation, realized by oxygen hollow fibers inside the bioreactor between the matrix layers, assuring optimal oxygenation of the hepatocytes without large oxygen and bicarbonate gradients in the bioreactor.

The first bioreactors were hand made with a total extra-fiber space of 11 ml (diameter 1.7 cm, length 15.5 cm) (Fig. 1). Hepatocyte seeding on the non-woven polyester matrix in the extra-fiber space was achieved by injecting the cell suspension via the side ports of the bioreactor. The same ports were used for medium perfusion after cell immobilization. Flow-sensitive magnetic resonance imaging was performed of the bioreactor to show equal flow distribution through the bioreactor.

Figure 1. Schematic drawing of a transverse and longitudinal cross section of the small-scaled bioreactor. The system is composed of a polysulfone housing (A) comprising a three-dimensional non-woven polyester matrix (B) for high-density hepatocyte culture and hydrophobic polypropylene hollow-fiber membranes (C) for oxygen supply and carbon dioxide removal. Medium is perfused through the extra-fiber bioreactor space via the side ports (F) and is in direct cell contact. The bioreactor is perfused with culture gas via the endcaps (E). The hollow fibers act as spacers between the layers of the 3D matrix, creating numerous channels or extra-fiber space (D).
vitro bioreactor function tests were performed after loading $2 \times 10^6$ viable porcine hepatocytes in the 11-ml bioreactor.\(^\text{14}\)

Hepatocytes were isolated from porcine livers using a simple two-step collagenase perfusion technique originally described by Seglen\(^\text{16}\) and modified by te Velde et al.\(^\text{17}\)

Trypan blue viability of the cells varied between 71 and 96%. Hepatocytes in the bioreactor were cultured in a recirculating culture medium (flow 5 ml/min) at 37°C and oxygenated by culture gas (95% air and 5% CO2). The bioreactor function was tested during 180 min on 3 consecutive days in a specified test medium. This small-scale bioreactor proved to eliminate galactose, synthesize urea out of ammonia and metabolizes lidocaine via the cytochrome P450 enzyme system. Furthermore, changes in amino acid status and production of proteins were shown. Microscopic evaluation of the hepatocytes on the polyester matrix showed viable hepatocyte aggregates after 5 days of culture (Fig. 2). In a

![Figure 2. a Light microscopic photomicrograph of a cross section of the 3D matrix from a small-scaled bioreactor in which $2 \times 10^6$ viable porcine hepatocytes/ml had been cultured for 5 days. The hepatocytes form small aggregates which immobilize on and between the polyester fibers (translucent circles, 13 \text{\textmu}m diameter) of non-woven fabric. b Scanning electron micrograph of isolated porcine hepatocytes cultured for 5 days in the 3D matrix of a small-scaled bioreactor. Small hepatocyte aggregates entrapped between the polyester fibers of the non-woven polyester matrix are shown. Tentacles, possibly of protein origin, cleave the inter-fiber space.](image-url)
Chapter 2

In a previous study, the functional activity of porcine hepatocytes cultured with several extracellular matrix constituents was tested. With the exception of Matrigel, neither of the extracellular matrix contents enhanced hepatocyte function. However, Matrigel was not further used because the presence of mouse sarcoma material was considered to be unacceptable in clinical application of the BAL.\textsuperscript{17}

After testing the small-scale bioreactor, the next step was to develop a large-scale BAL system sufficient to treat patients with ALF. It was estimated that 10–20 \times 10^9 hepatocytes or 100–200 ml hepatocyte pellet volume is needed in BAL systems to treat ALF patients\textsuperscript{18}, a cell amount factor 500–1,000 more than used in the small-scale bioreactor.

The first-generation large-scale bioreactor (Fig. 3a) consisted of a polysulfone housing with a volume of 420 ml harboring a three-dimensional non-woven polyester matrix, which is circularly wound around a massive polysulfone core (for technical details, see Table 1). Hydrophobic polypropylene gas capillaries are positioned in a parallel fashion between the layers of the polyester matrix. The ends of the oxygen capillaries are embedded in polyurethane resin using dialyzer potting techniques, and fitted with gas inlet and outlet caps. Gas in and outflow ports (G, Fig. 3b) are situated at the side of the bioreactor. According to function tests used for the small-scaled bioreactor, the large-scaled bioreactor was tested \textit{in vitro} with 10 \times 10^9 viable hepatocytes and showed initially good biochemical function.\textsuperscript{19} However, the function per hepatocyte was

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Transmission electron micrograph of a hepatocyte aggregate from the 3D matrix after 4 days of culture in a bioreactor (bar represents 1 \textmu m). Neighboring cells reconstitute bile canaliculus-like structures (BC) with typical microvilli and junctional complexes including tight junctions (arrows) and desmosomes (D). Other cell structures displayed are: mitochondria (M), Golgi complexes (G), rough endoplasmatic reticulum (RER), a peroxisome (P) and a nucleus (N).}
\end{figure}
drastically reduced and decreased to 25% after 3 days of culture. This failure in adequate scaling up of the bioreactor depended on: (1) poor hepatocyte distribution throughout the bioreactor; (2) inadequate positioning of the oxygen fibers; (3) diminished hydrodynamic features with insufficiently perfused areas, and (4) areas in the bioreactor without matrix where hepatocytes clump together in large cell volumes and are insufficiently perfused and oxygenated. In addition to these disadvantages of the device, hepatocyte isolation and culturing conditions needed to be improved. Therefore a second-generation large-scale bioreactor was developed together with improved hepatocyte isolation techniques and culture conditions. The second-generation bioreactor (manufactured by RanD Srl, Medolla, Italy) is a modified version of the first-generation bioreactor (for details, see Fig. 3b and Table 1). The most important improvements consist of: (1) complete visibility inside the device; (2) less tight configuration of matrix windings; (3) less dead space and an extra cell loading port that improves cell distribution, and (4) the change of gas capillary constitution into co-extrusions of polypropylene and polymethylpentene. The total extra-fiber volume of the second-generation bioreactor is 570 ml. The function of the second-generation bioreactor, loaded with $10 \times 10^9$ viable porcine hepatocytes, was improved by a factor 4–10, depending on the parameter measured, compared to the
first-generation bioreactor. Furthermore, bioreactor function after 3 days was about 80–90\% compared to the first day of culture.\cite{18} Also, technique of hepatocyte isolation and hepatocyte culture has been improved.\cite{18}

### Table 1. Technical features of AMC-BAL bioreactors; first vs. second generation bioreactor.

<table>
<thead>
<tr>
<th>Configuration</th>
<th>First generation bioreactor</th>
<th>Second generation bioreactor</th>
<th>Unit</th>
<th>Delta %</th>
</tr>
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<tr>
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<td>IN-OUT connection inter-axe</td>
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<td>mm</td>
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<td>Material</td>
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<tr>
<td>Inlet/Outlet plasma connector</td>
<td></td>
<td></td>
<td>mm</td>
<td></td>
</tr>
<tr>
<td>Material</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Housing</td>
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<tr>
<td>Material</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Potting to potting cut-high</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Material</td>
<td></td>
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<tr>
<td>IN-OUT connection inter-axe</td>
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<tr>
<td>Inlet/Outlet plasma connector</td>
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<td>Polysulfon</td>
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<td><strong>Non woven Matrix</strong></td>
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<tr>
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</tr>
<tr>
<td>Polyester fabric</td>
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<td>Useful height</td>
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<td>5800</td>
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<td>Thickness</td>
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<td>400</td>
<td>μm</td>
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<tr>
<td>Useful surface for cells seeding</td>
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<td>cm²</td>
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<td>Hydrophilic surface treatment</td>
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<td><strong>Oxygenator fibres</strong></td>
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<td>Heating system for gas</td>
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In vivo Studies

In a review paper on animal models of ALF, recommendations were given for treatment design. Small as well as large animal studies were performed with the AMC-BAL. Flendrig et al. tested the first small-scale bioreactor (11 ml volume) loaded with $440 \times 10^6$ porcine hepatocytes with a trypan blue viability of 74–85% in rats with complete liver ischemia (LIS). Treatment was started 30 min after initiation of LIS. A significant prolongation of survival time was found: treated vs. control group, $11.0 \pm 2.2$ vs. $5.1 \pm 1.7$ h ($p < 0.001$) respectively. The treated group showed significantly delayed deterioration in clinical signs of hepatic encephalopathy. Significantly reduced ammonia and lactate serum levels were found in the treated group in comparison to the control groups.

After this success in the small animal studies, two large animal studies were performed using two different ALF models. First, LIS pigs (weighing about 35 kg) were treated with the AMC-BAL (first-generation 420 ml volume) loaded with approximately $14 \times 10^9$ viable porcine hepatocytes. The AMC-BAL treatment of LIS pigs started 6 h after inducing total LIS, and was continued for 24 h. Significantly prolonged survival time ($51 \pm 3$ h) was observed compared to control groups ($33 \pm 3$ h). Furthermore, BAL treatment showed lower ammonia and bilirubin levels in the BAL-treated pigs compared to control groups.

The LIS pig model was followed by a study in anhepatic pigs. This model is based on total hepatectomy with restoration of caval continuity using a polyethylene, three-way prosthesis. The BAL system (first-generation large-scale bioreactor) was loaded with approximately $10 \times 10^9$ viable autologous porcine hepatocytes, isolated from the explanted liver and cultured in a culture medium prior to treatment. Twenty-four hours after hepatectomy, BAL treatment was started and continued for 24 h. Survival of the anhepatic pigs was significantly increased in the BAL-treated group ($65 \pm 15$ h), as compared to the control groups ($46 \pm 6$ h) ($p = 0.02$). A 24-hour lasting BAL treatment prolonged life in the anhepatic pig for about 20 h. Mean blood ammonia levels during BAL treatment were significantly lower in the BAL-treated group in comparison to control groups. To examine synthesis of coagulation factors by the AMC-BAL, several coagulation and anticoagulation parameters were measured. Factors II, V, VII, AT-III and fibrinogen rapidly decreased after total hepatectomy in pigs in accordance with the anhepatic state of the animals. Factor VIII levels were not influenced by the hepatectomy, indicating extrahepatic synthesis of factor VIII. A mild drop in platelet count was seen in all groups. Treatment of anhepatic pigs with the AMC-BAL based on freshly isolated porcine hepatocytes did not result in an improved coagulation state, due to extensive consumption of clotting factors in this model with large surgical wounds. However, increased levels of TAT complexes and prothrombin fragments F1 + 2 during treatment
of anhepatic pigs indicate synthesis and direct activation of coagulation factors, leading to thrombin generation. Hence, this study demonstrated that the porcine hepatocytes in the AMC-BAL were capable of synthesizing coagulation factors.

No important side effects of BAL treatment were observed in both in vivo studies. In view of the successful outcome in survival, clinical and biochemical parameters, we were encouraged to start a phase I study in ALF patients.

**Logistics of BAL Treatment**

Apart from which cell type to be used in clinically applied BAL systems, the assurance that each ALF patient will receive the bioreactor with the highest function is a prerequisite for optimal treatment. In general there are two options to provide the hospitalized patient with a BAL system: (1) transportation of an empty bioreactor separate from the hepatocytes (which are usually cryopreserved in stock) and charging the BAL on demand in the local hospital\(^{26,27}\), or (2) assembling the bioreactor in a specialized laboratory and transport the charged BAL to the patient on demand\(^{28,30}\).

The main advantages of the first option are the direct availability of BAL components in the hospital and the possibility to decide at any moment whether the BAL system has to be assembled or not. Of course, one should realize that testing function and sterility of the assembled BAL is a prerequisite before application in the patient. An important disadvantage is the requirement of special equipment and a certified laboratory environment for thawing cryopreserved hepatocytes and loading a bioreactor for clinical purposes. The second option has the advantage that loading and testing of the bioreactor can be carried out under controlled conditions in a centralized and well-equipped laboratory with qualified personnel. An additional advantage is that fresh hepatocytes can be used instead of cryopreserved hepatocytes that after a freezing-and-thawing cycle are seriously affected with regard to their functionality\(^{31,32}\). The disadvantages are the delay in delivery

![Figure 4. Airdrive disposable perfusion system.](image)
at the bedside of the patient and, more importantly, the risk that undefined transport conditions lead to loss of function. One option is to transport the cell loaded bioreactor in an organ preservation solution at 4°C. However, transport of a cold preserved BAL system in Celsior negatively affects cell viability and hepatocyte-specific functions. An alternative is to transport the BAL system under oxygenation and medium perfusion at (sub)normothermic temperatures. The effect of subnormothermic preservation on freshly isolated porcine hepatocytes was assessed in an in vitro monolayer hepatocyte culture model under oxygenation. The conclusion of this study was that freshly isolated porcine hepatocytes can be preserved for 24 hours at subnormothermic temperatures as low as 15°C on monolayer. In a second study, the feasibility of preserving the complete AMC-BAL at mild hypothermic temperatures was assessed. The conclusion was that mild hypothermic preservation at temperatures as low as 15°C and for a duration of 24 hours is a feasible method to preserve BAL systems, i.e. AMC-BAL, loaded with freshly isolated porcine liver cells. These studies indicate that the logistics of BAL transport from the laboratory to the hospital will be simplified. Transport of the AMC-BAL can be realized by the Airdrive system as has been developed in the AMC by Doorschodt et al. (Fig. 4). The key component of this system is the air-pressure-driven perfusion pump. This system can simultaneously perfuse and oxygenate the AMC-BAL under sterile conditions for a transport period of 24 h.

Phase I Study

At present, eleven BAL systems have found clinical application. Three systems were studied in controlled clinical trials in ALF patients, but showed no significant improvement of survival when analyzed by intention to treat. The other systems were studied in phase I trials or as treatment of a single patient. All showed to be safe and most of them resulted in improvement of clinical and/or biochemical parameters. A phase I trial in ALF patients has been performed in Italy because treatment of patients with xenogeneic material (among which porcine hepatocytes) is prohibited by law in the Netherlands, as in several other European countries. In two Italian hospitals, Cardarelli Hospital, Naples, and St. Eugenio Hospital, Rome, 12 ALF patients were included in a phase I study with the AMC-BAL (first-generation bioreactor, loaded with approximately $10 \times 10^9$ viable freshly isolated porcine hepatocytes). All patients had hyperacute ($n = 11$) or acute ($n = 1$) liver failure according to Crepaldi et al. and met the criteria for OLT. All patients had grade III–IV encephalopathy. The cause of ALF in six patients was acute hepatitis B. Other patients had acute hepatitis A, Wilson’s disease, intoxication or acute fatty liver of pregnancy. In two patients the cause of liver failure was not elucidated. Total duration of AMC-BAL treatment ranged from 4 to 35 h.
Three patients received serial treatment with two BALs. Eleven of the 12 patients were successfully bridged to OLT. One patient recovered after two BAL treatments over an interval of 3 days without needing OLT. Four patients died of complications post-OLT. One-year survival, post-OLT was 67%. Treatment of all patients was associated with an improved neurological state and stabilization of hemodynamics. Increased diuresis was noticed in patients with renal insufficiency. After AMC-BAL treatment, the average plasma ammonia and bilirubin levels decreased by 44 and 31%, respectively. No major adverse events were observed during BAL treatment. In two patients a short transient period of hypotension was observed after connection to the BAL system. This hypotension was readily corrected by dopamine and fluid administration and BAL treatment could be continued.

One of the largest concerns of xenotransplantation is the danger of zoonosis. In our phase I study, we used hepatocytes isolated from specified pathogen-free pigs (Institute Zooprofylactico, Brescia, Italy). Before BAL treatment, the hepatocyte-loaded bioreactors were tested for bacterial and fungal contamination using cultures and a PCR bacterial DNA test, ready within 4 h after sampling. The fear of zoonosis concerns especially the danger of retroviruses. Endogenous retroviruses are part of the porcine genome and may potentially infect the treated patient. If such a retrovirus mutates into a virulent one it may theoretically introduce a new infectious disease in humans. We have tested porcine endogenous retrovirus (PERV) in our BAL-treated patients with in vitro and in vivo assays. In these studies, only PERV-DNA was found in patients’ plasma directly after BAL treatment and had disappeared shortly thereafter. No PERV-RNA could be detected in plasma or peripheral blood mononuclear cells during a follow-up of 2 years. In addition, during this follow-up, no signs or symptoms of a possible new disease were observed in these patients. The advice is to survey patients’ plasma for PERV after treatment with porcine hepatocytes in a BAL for at least 6 months, because viremia after exposure to other retroviruses is mostly seen within this period. Patients should be controlled lifelong for possible signs or symptoms of known and unknown diseases. Despite accumulating evidence, showing safety of porcine hepatocyte based BAL systems, the need for human hepatocytes remains. An important advantage of using human hepatocytes is the absence of possible immunological reactions and the possibility of long-term and recurrent treatment.

**Human Cell Lines**

Most bioartificial livers are developed and tested with the use of freshly isolated porcine hepatocytes, but apart from xenotransplantation-related risks, logistical, ethical and quality control considerations urge the use of human cells for future BAL application. Ideally
these cells should exhibit liver-specific function comparable to primary freshly isolated hepatocytes, they should have the capacity to be expanded in a laboratory setting and they must have proven to be safe for BAL application. Freshly isolated human hepatocytes would be a very attractive cell source because of their excellent hepatic function. Primary human hepatocytes can be isolated from discarded donor livers, but availability is scarce, function is diminished and the proliferation capacity of primary hepatocytes in vitro is very limited.

To obtain cell quantities appropriate for large-scale bioreactor production, many groups tried to develop a human hepatocyte cell line showing both proliferative capacity and hepatic function. However, these two features are hard to combine at the same time in vitro.

One of the first explored options was the use of cell lines originating from human hepatoblastoma or hepatocellular carcinoma. These cell lines show excellent in vitro proliferation capacity, but in general, hepatic functions are decreased. One of the best-characterized tumor-derived hepatic cell lines is HepG2, isolated from a human hepatoblastoma. This cell line has been tested in a gel entrapment BAL and displayed hepatic functions such as ureagenesis, glucuronidation and sulfation, but only at approximately 10% of the function of freshly isolated rat hepatocytes. Moreover, no cytochrome P450 activity was detectable. In one experiment it was shown that tumor nodules in the extracapillary compartment of the BAL occurred after 60 days of culture, which raises concerns about the risk of tumor transmigration to the patient.

A clonal derivative of HepG2, C3A, was selected for strong contact inhibition of growth and high albumin and alpha-fetoprotein production and is the only cell line that has been applied in a BAL device to treat patients in a pilot-controlled clinical trial. However, treatment did not improve survival of the patients.

As an alternative for tumor-derived hepatic cell lines, cell lines have been established by in vitro immortalization of human hepatocytes. Most cell lines have been developed by introduction of genes that stimulate cell cycle progression like cyclin D1, E2F or inhibitors of cell cycle controlling factors p53 and pRb, like the viral oncogene simian virus 40 large T antigen (SV40T). In addition, reconstitution of telomerase activity has been applied. Although several immortalized cell lines have been reported (IHH-A5, HepZ, THLE-2&3), in vitro immortalization of mature human hepatocytes is a rare event, in which the role of spontaneously occurring mutations that contribute to immortalization, but also to transformation, cannot be excluded. Again these cell lines generally exhibit high proliferation capacity combined with low hepatic function, probably due to the fact that in vitro hepatic function and proliferation cannot take place at the same time. To overcome this problem, immortalization can be made conditional by placing the immortalizing gene in a controllable expression system. Kobayashi et al. introduced the NKNT-3 cell line in which the Cre-LoxP system is used to conditionally immortalize mature human
hepatocytes with the SV40T gene. In this system the immortalizing gene can be excised from the genome, resulting in inhibition of proliferation and induce induction of hepatic differentiation. Indeed, excision of the SV40T gene leads to induction of expression of albumin, bilirubin-uridine diphosphate-glucuronosyltransferase, glutamine synthetase, π class glutathione S-transferase and human blood coagulation factor X. Transplantation of NKNT-3 cells rescues 90% hepatectomized rats. However, we found that the levels of hepatic function of NKNT-3 cells in vitro are too low to be useful for BAL applications.

We attempted to immortalize adult human hepatocytes ourselves, using human hepatocytes from more than 10 different donors, by different immortalizing genes and different methods of gene transfer. Although the cells could be kept in culture for up to 4 months, their proliferation capacity was negligible. In our view, the proliferation capacity of mature hepatocytes is too low to allow immortalization without drastically interfering with the cell cycle control, thus leading to transformation of cells, concomitant loss of functionality and risk of tumorigenicity.

Due to the limited success of establishing cell lines from mature hepatocytes, studies on other cell sources with more proliferation capacity, such as embryonic stem cells or fetal cells, are accumulating. Animal studies show that cells from embryonic mouse liver can proliferate in vitro and contribute to liver parenchyma when transplanted in mice with a genetic liver disorder, suggesting a potential for hepatic differentiation. However, in vitro these cells display low initial hepatic functionality and therefore stimulation of hepatic differentiation in vitro is becoming increasingly important. In vivo, a complex system of soluble factors and cell-cell interactions is responsible for the differentiation of embryonic or fetal cells into mature hepatocytes and we are only at the start of unravelling this system. More insight in the processes that stimulate hepatic differentiation in vivo will lead to the development of in vitro conditions to increase the hepatic functions of hepatic cell lines and to make these applicable for BAL applications.

End Note

Although the first dialysis machine for replacement of kidney function was conceived in 1941, a few years later followed by the artificial heart, an effective (bio)artificial liver has not become available for routine use until this day. The most important obstacle, which at the same time is the most vital component of any BAL device, is the biocomponent. This must represent a viable and functioning hepatocyte cell mass, composed of either primary hepatocytes (10–20 billion cells) or a hepatocyte cell line. The good news is that high-performance bioreactors have been developed and that porcine primary hepatocytes loaded in a BAL have shown to be efficacious in prolonging survival in large animals with ALF.

End Note
and have successfully bridged ALF patients with grade III/IV coma to transplantation. The bad news is that we cannot use hepatocytes of porcine origin in many European countries, because of the theoretical risk of viral transfection. The future of BAL research therefore lies in the availability of alternative cell sources such as immortalized human hepatocyte cell lines – most likely derived from human fetal hepatocytes or human stem cells\(^5\) – and co-cultures of hepatocytes with other (liver) cells. Finally, these cells should be tested in BALs used in a randomized, controlled clinical trial in ALF patients. Only then we will know that four decades of painstaking research in BALs has been worthwhile.

**Reference List**

Chapter 2


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