Bioartificial liver support: developments in hepatocyte culture and bioreactor design

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Bioartificial liver devices aim to support patients with acute liver failure (ALF) until orthotopic liver transplantation (OLT) or spontaneous recovery due to hepatic regeneration can occur. However, initial clinical experiences with two devices have indicated that functional efficacy in this setting may be less than in the experimental situation. Several fundamental issues remain unresolved, including the cell mass required to provide meaningful support and which of those hepatocyte components and bioreactor designs so far proposed is best able to do this. In particular, further studies of the efficacy of devices incorporating human hepatocyte lines transformed by either cultural conditions or genetic engineering and those based on multi-channel or flat bed bioreactor designs in which hepatocytes are co-cultured with non-parenchymal cells are awaited. Controlled trials on a multicentre basis in well-defined patient groups and with standardised outcome measures will be required to properly evaluate the clinical value of these devices. A better understanding of factors promoting recovery and regeneration of the native liver and to what extent these can be provided by extracorporeal devices will be essential to the further development of effective bioartificial liver support systems.

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Acute liver failure (ALF) is a medical emergency which, despite improvements in modern intensive care, still carries a substantial mortality rate. In the most severe cases, urgent orthotopic liver transplantation (OLT) currently represents the only chance for survival. However, the supply of donor organs is limited and an organ may not become available in time. An effective temporary liver support system would improve the chance of survival in this circumstance by sustaining patients until a donor liver becomes available. Furthermore, the known capacity of the native liver to regenerate following recovery from ALF, as further demonstrated by recent experience with the technique of auxiliary partial OLT, raises the possibility that the use of temporary liver support for a sufficient period of time may even obviate the need for OLT in at least some cases.

Early attempts at providing temporary liver support involved various extracorporeal systems, including whole liver perfusion, cross circulation, haemodialysis, charcoal haemoperfusion and plasma exchange.
Most were based on the premise that removal of water soluble and albumin bound toxins was of paramount importance, as such compounds were thought to be responsible for cerebral oedema and other manifestations of ALF. The best studied example of a completely artificial system is charcoal haemoperfusion, the initial promising reports of which in both experimental animal models and the clinical setting could not be substantiated in a subsequent controlled clinical trial. Consequently, it became generally assumed that ‘bioartificial’ liver support systems, with extracorporeal bioreactors containing hepatocytes providing additional synthetic and biotransformatory liver functions, may be more effective than completely artificial systems which provided excretory capacity alone.

The increasing recent interest in this field has resulted from the availability of various transformed hepatocyte lines, the development of more effective matrix support for hepatocyte growth and function in culture and the introduction of innovative bioreactor designs in an attempt to more closely simulate *in vivo* liver architecture and further extend the functional lifetime of the device. Several bioartificial liver support systems have yielded promising results *in vitro* and in experimental animal models of ALF. Preliminary clinical experience with two of these has also been reported.

**Hepatocyte component**

It is generally considered that about 20–40% of normal hepatocyte mass is required to sustain life. On this basis, the bioreactor would need to incorporate a large mass of functioning hepatocytes, in the order of 300 g, for the adequate support of an adult with ALF. However, the reliability of this assessment of the amount of hepatocytes required is complicated by the fact that the severity and duration of ALF vary according to its aetiology and evidence that surviving hepatocyte volume fractions determined by liver biopsy of >40% do not necessarily portend a favourable prognosis.

Cell lines which have been employed or are currently undergoing investigation for use in bioartificial liver support systems include primary hepatocytes isolated from human or animal livers, and various transformed human cells, such as hepatoma, hepatoblastoma and immortalised hepatocyte lines. Significant differences in the functional activities of these cells have been described. However, the impact of these differences on the clinical efficacy of the support device is currently unclear, since the pathophysiology of ALF is incompletely defined and, in particular, those specific excretory, metabolic and biotransformatory...
activities primarily responsible for patient survival and native liver regeneration are poorly understood.

Primary human hepatocytes are mainly obtained from resected surgical specimens, from unused segments of donor organs for OLT or from the explanted livers of patients undergoing OLT for disorders such as localised tumour. There is, at present, no reliable method of isolating a satisfactory number of viable hepatocytes from needle biopsy-sized fragments of liver. Consequently, the availability of these cells is limited. Indeed, neither of the devices of which there is preliminary clinical experience at present employ primary human hepatocytes. Conversely, the supply of primary mammalian, especially porcine, hepatocytes is almost unlimited. Inter-species differences in plasma proteins are not necessarily problematic, since deficient plasma levels of clotting factors, for example, can usually be adequately replenished by infusion of fresh frozen plasma or specific protein concentrates.

Whether inter-species differences in cytochrome P450 and other enzyme activities are important limitations when animal cells are used is unknown. Although pigs have been considered not to harbour infective agents which could cause disease in humans, a porcine endogenous retrovirus resistant to lysis by human complement and capable of infecting human cells in vitro has recently been reported. Indeed, all clinical xenotransplants have now been banned in the UK until further studies addressing the infectious disease risks can be undertaken. The implications for the use of porcine hepatocytes in extracorporeal systems will require clarification.

Irrespective of their source, a limiting problem with the use of primary hepatocytes has been the rapid loss of differentiated function in culture, although this may be prolonged to some extent as a result of advances in matrix support and bioreactor design, as discussed later. A novel, alternative approach to generating proliferating hepatocyte lines which maintain their differentiated function in culture is to introduce into normal human hepatocytes a temperature-sensitive variant of the SV40 large-T antigen gene. This binds to the cell cycle control protein, p53, and produces cell lines which proliferate at 33°C but cease proliferating and develop enhanced differentiated function at 39°C. Several non-clonal human hepatocyte cell lines have recently been successfully infected with an amphotrophic mouse retrovirus containing the SV40 large-T antigen gene and are undergoing further characterisation. Another hepatocyte line derived from normal human liver, HHY41, has also been shown to proliferate freely and maintain its differentiated function in culture for prolonged periods. The HH25 cell line, which like HHY41 cells has been immortalised from normal hepatocytes by virtue of the conditions in which it was originally cultured without the use of oncogenes or carcinogens, also proliferates.
in culture and expresses differentiated function comparable to that of HHY41 cells\textsuperscript{20}.

Transformed human hepatoma or hepatoblastoma cell lines, such as Hep G2 and C3A cells, are cheaper to obtain and easier to maintain in culture than primary hepatocytes, as a result of their less stringent metabolic requirements\textsuperscript{22}. However, the main problems have been a reduced level and restricted range of differentiated function compared to normal hepatocytes. Direct comparisons of the functional capacities of primary hepatocytes and Hep G2 cells under bioreactor conditions have demonstrated that gluconeogenesis, lactate metabolism, ureagenesis, glucuronidation, sulphation and oxidation are each significantly reduced in the transformed cell line\textsuperscript{23,24}. Cytochrome P450 activity is incomplete and specific reactions, such as the acute phase response to interleukin-6, are also deranged\textsuperscript{13}. Although similar concerns have been expressed concerning the functional activity of C3A cells\textsuperscript{13}, others have reported normal ureagenesis, gluconeogenesis and inducible cytochrome P450 activity\textsuperscript{25}. The risk of tumour cell propagation from bioreactors containing tumour cell lines, as reported under experimental conditions\textsuperscript{23}, is prevented in the clinical setting by the incorporation of filters which prevent detached cells from escaping downstream\textsuperscript{25}.

**Matrix support**

Stationary suspension culture of isolated hepatocytes is ineffective, since in this circumstance hepatocytes lose differentiated function within hours\textsuperscript{26}. An alternative technique resulting in improved cell viability and functional activity is attachment culture. Using this approach, hepatocytes are grown either in suspension as self- or microcarrier-induced multicellular aggregates or attached to various semi-permeable, usually hollow fibre, membranes which feature high surface area to volume ratios. Porous synthetic polymer hollow fibres seem particularly promising surfaces for cell attachment, although they are yet to be fully validated under bioreactor perfusion conditions\textsuperscript{27,28}. Whilst standard monolayer culture conditions are sufficient for supporting cell viability for up to 1–2 weeks\textsuperscript{29}, hepatocytes lose differentiated function after only a few days\textsuperscript{30}. Conversely, the various modifications discussed below have been shown to sustain differentiated function in vitro for at least 2–3 weeks\textsuperscript{31,32}. For example, use of an overlying layer of collagen gel, a collagen gel sandwich or cylindrical collagen gel entrapment is an important modification which has been shown to be advantageous\textsuperscript{33,34}, possibly at least in part by
abrogating the dedifferentiation-inducing effect of hepatocyte growth factor, high levels of which are found in the plasma of patients with ALF. Co-culture of hepatocytes with extracellular matrix and non-parenchymal cells also improves the performance of hepatocytes in culture.

It has become increasingly clear that three dimensional rather than monolayer growth is particularly important for maintaining differentiated hepatocyte function in culture. One means of establishing three dimensional hepatocyte growth is the creation of multicellular spheroid aggregates. Early methods utilising stationary culture techniques had the disadvantage of requiring several days for spheroid formation, rendering this approach impractical for clinical use. However, more recent work has shown that multicellular spheroids can be rapidly created by rotational culture systems or by incubation with a small number of collagen-coated dextran, polystyrene or glass microcarriers as a nidus for cell aggregation. On transmission electron microscopy, these matrix-induced hepatocyte aggregates have an ultrastructure resembling that of a normal liver, with maintenance of cell polarity, gap junctions, bile canaliculi and intact organelles and glycogen granules.

Cell encapsulation within alginate-polylysine, polyacrylate or cellulose acetate also favours the development of three dimensional hepatocyte growth. The potential for three dimensional structure formation can be further enhanced by enriching the solution with natural extracellular matrix proteins prior to encapsulation. Co-culture with non-parenchymal cells within the capsule also significantly enhances hepatocyte viability and function, possibly due to the mixed effects of autocrine and paracrine stimulation by growth factors derived from the non-parenchymal cells and the production of extracellular matrix. Improvements in the reproducibility of the polymer components and refinements in membrane characteristics may further enhance the efficacy of this technique. Fibrin deposition as hepatocytes condition their environment in culture may also promote three dimensional growth, as recently demonstrated with both microcarrier-attached and freshly trypsinised HHY41 cells within a hollow fibre cartridge.

Cryopreservation of freshly isolated hepatocytes is notoriously difficult, with considerable loss of viability invariably occurring after thawing. By contrast, encapsulated and microcarrier-attached hepatocytes and gel-entrapped hepatocyte cultures have been reported to maintain their functional activity following cryopreservation. This is an important consideration given the logistic difficulties otherwise associated with storage and transportation of viable cells to the patient.
Bioartificial liver support

Bioreactor design

Some bioreactor designs feature cylinders, including those packed with glass beads for hepatocyte attachment, through which blood is perfused. Such a packed bed bioreactor containing primary hepatocyte spheroid aggregates has been shown to support hepatocyte viability in culture for at least 15 days, as assessed by albumin and urea synthesis rates and cell morphology. An alternative approach is based on the inclusion of a semi-permeable membrane. These designs each feature at least one compartment where blood from the patient’s circulation flows and another where cells are cultured either in suspension or in adhesion to the membrane. The rate of transfer of oxygen, nutrients, toxins and other factors within the device depends on the fluid dynamics of both the blood and the cell compartments, the transport properties of the membrane and cell-membrane configurations. Additional compartments may be incorporated for cell nutrition, cell oxygenation, carbon dioxide and toxic metabolite removal and, possibly, the delivery of factors promoting native liver regeneration from the bioreactor to the patient’s blood stream. In addition, these membranes protect liver cells from shear damage within the bioreactor. The isolation of immunocompetent proteins and cells is attained with membranes with molecular weight cut-off ranging from 50–100 kDa, thus enabling the use of xenogeneic or allogeneic cells without the need for immunosuppression.

With regard to hollow fibre membranes, cells may be cultured either inside or outside the lumen. Culture of hepatocytes entrapped within a gel bed within the lumen of hollow fibre membranes permits a better cell distribution than that attained with non-entrapped cells. However, solute transport in the gel bed occurs purely by diffusion, raising the possibility of steep concentration gradients and hence increased mass transfer resistance, which may result in cell starvation or the accumulation of toxic metabolites near the cell surface. Culture of self- or microcarrier-induced hepatocyte spheroid aggregates in suspension outside the hollow fibre membranes has alternatively been proposed. In this design, blood flows through the hollow fibre lumen. However, the liquid stagnant layer in the cell compartment increases the diffusion path to the cells, and hence the mass transport resistance. The outer surface of the membranes may alternatively be used for cell adhesion, although solutes have to be transported in the cell compartment through a large mass of cells in this circumstance, again interfering with the efficacy of mass transfer.

Several innovative membrane-based bioreactor designs have recently been proposed, including that by Gerlach et al, which features porcine hepatocytes cultured in adhesion on the outer surface of and between four interwoven, biomatrix-coated hollow fibre membrane systems.
arranged in three planes at 90° to each other and which serve independent functions. This design incorporates sinusoidal endothelial cell co-culture and allows decentralised cell perfusion and the independent supply of oxygen and nutrients and metabolite exchange at low gradients. Solute transport through the cell mass is appreciably enhanced. Hepatocytes spontaneously form aggregates and assume a three dimensional structure during culture within the bioreactor and have been shown to express differentiated function in vitro for up to 4 weeks.

A bilaminar flat bed configuration proposed by Bader et al⁹ represents another three dimensional co-culture model, designed to mimic the sinusoid-hepatocyte relation and interactions between parenchymal and non-parenchymal cells in vivo. The design, as tested experimentally, includes rat hepatocytes in monolayer culture within a collagen gel sandwich overlying porous silicon-coated membranes. The second collagen layer is overlaid not only with sinusoidal endothelial cells but also other non-parenchymal cells, including Kupffer cells and Ito cells. Increased albumin and urea synthesis for up to 8 weeks and increased cytochrome P450 activity at 1 week compared to non-overlaid designs have been reported in vitro⁵⁰,⁵¹.

On the premise that a device allowing more direct contact between hepatocytes and perfused blood would allow improved mass transfer capacity compared to hollow fibre bioreactors, yet another design has been proposed by Naruse et al⁵², in which porcine hepatocyte spheroids are immobilised on non-woven polyester fabric. The non-woven fabric module expressed better metabolic and synthetic functions at 24 h than a hollow fibre module containing spheroids in suspension culture, possibly because the former could accommodate more hepatocytes per unit volume. Longer term results are not yet available and the immunoexclusion properties of this fabric have not been addressed.

**Results in experimental animal models**

Several of the prototype devices described above, including those based on hollow fibre, flat plate, packed bed or other cylindrical bioreactor designs, have now undergone assessment in both small (rodent and rabbit) and large (porcine and canine) animal models of ALF. Results obtained with some devices which have not yet undergone clinical evaluation²⁴,⁵²-⁶¹ are listed in Table 1.

Despite differences in bioreactor design, these devices have in common the incorporation of animal, usually porcine, cells as the hepatocyte component, either microencapsulated, gel-entrapped, attached to
Table 1  Experience in animal models of acute liver failure (ALF) with some bioartificial liver designs which have not yet undergone clinical evaluation

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal model of ALF</th>
<th>Hepatocyte component</th>
<th>Bioreactor design</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shnyra et al. 53</td>
<td>Rodent; D-galactosamine</td>
<td>Porane, microcarrier-attached</td>
<td>Unspecified cylinder</td>
<td>Significantly improved survival in treated (12/20, 60%) compared to control rats (1/20, 5%) at 48 h</td>
</tr>
<tr>
<td>Duct et al. 54</td>
<td>Porane, D-galactosamine</td>
<td>Porane, microencapsulated</td>
<td>Packed bed</td>
<td>Significantly improved survival in treated compared to control groups. Survival significantly prolonged even in animals with more severe liver injury, as assessed by peak AST levels</td>
</tr>
<tr>
<td>Sieloff et al. 53</td>
<td>Canine, D-galactosamine</td>
<td>Porane, collagen gel-entrapped</td>
<td>Hollow fibre</td>
<td>Significantly delayed rise of lactate and ammonia and onset of coma and improved survival in 5 treated dogs compared to controls over 48 h</td>
</tr>
<tr>
<td>Nagaki et al. 54</td>
<td>Porcine, acute ischaemia</td>
<td>Porane; basement membrane gel-entrapped</td>
<td>Hollow fibre</td>
<td>Improved haemodynamic stability and longer survival (12.5 ± 3.5 h) in 3 treated pigs compared to a control (1.5 h)</td>
</tr>
<tr>
<td>Sheil et al. 56</td>
<td>Porcine, acute ischaemia</td>
<td>Porane, microcarrier-attached</td>
<td>Hollow fibre</td>
<td>Significantly less ammonia accumulation and reduced lactate levels in 5 treated pigs compared to controls</td>
</tr>
<tr>
<td>Chen et al. 57</td>
<td>Porcine, anhepatic</td>
<td>Porane aggregates</td>
<td>Hollow fibre</td>
<td>System contains charcoal adsorbent column, improved survival time (25 ± 7 h) in 6 treated pigs compared to controls treated with charcoal haemoperfusion alone (13 ± 2 h) or with perfusion through a system containing neither hepatocytes nor the charcoal column (17 ± 7 h)</td>
</tr>
<tr>
<td>Gerlach et al. 58</td>
<td>Porcine, anhepatic</td>
<td>Porane aggregates</td>
<td>Hollow fibre</td>
<td>Four independent membrane systems serve different functions, co-culture of hepatocytes with sinusoidal endothelial cells, treatment associated with delayed onset of encephalopathy and reduced blood ammonia levels</td>
</tr>
<tr>
<td>Naruse et al. 53</td>
<td>Porcine, unspecified</td>
<td>Porane aggregates</td>
<td>Non-woven polyester fabric</td>
<td>Reduced blood ammonia and bile acid levels and increased blood glucose concentrations compared to before treatment</td>
</tr>
<tr>
<td>Nyberg et al. 59</td>
<td>Rabbit; anhepatic</td>
<td>Rodent; collagen gel-entrapped</td>
<td>Hollow fibre</td>
<td>Treatment associated with increased albumin synthesis, reduced exogenous glucose requirements, normalisation of aromatic amino acid levels and evidence of sulphation, glucuronidation and cytochrome P450 activity</td>
</tr>
<tr>
<td>Jauregui et al. 60</td>
<td>Rabbit; D-galactosamine</td>
<td>Rabbit</td>
<td>Hollow fibre</td>
<td>Significantly increased survival, delayed onset of encephalopathy and increased native hepatocyte regeneration in 11 treated rabbits compared to controls</td>
</tr>
<tr>
<td>Uchino et al. 61</td>
<td>Canine, anhepatic</td>
<td>Canine</td>
<td>Multiple collagen-coated glass plates</td>
<td>Improved survival (55 ± 10 h) in 4 treated dogs compared to 6 untreated (21 ± 6 h) and 3 plasma exchange (28 ± 4 h) controls, lower serum ammonia levels in treated dogs</td>
</tr>
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</table>
microcarriers or as self- or matrix-induced hepatocyte aggregates and, on occasion, in co-culture with non-parenchymal cells. Although sample sizes have tended to be small, these studies have generally demonstrated not only good metabolic function of the device but also improved haemodynamic stability, delayed onset of encephalopathy or progression to coma and, importantly, a survival advantage, at least in the short term of up to several days.

In a number of studies, treatment was commenced at an early stage, raising the possibility that the beneficial effects may have been more related to the prevention of progression of the liver failure rather than its reversal. However, these experiences more than justify the continued research and development of suitably scaled-up devices for testing in humans. Assessment as to which devices should be prioritised for future clinical evaluation is at present hindered by the fact that no studies have directly compared their efficacy in the same experimental animal model of ALF.

Clinical experience with bioartificial liver support

Two bioartificial devices initially investigated in animal models of ALF have now undergone preliminary clinical evaluation. These devices differ from each other in several notable respects. In the ‘bioartificial liver’ (BAL)\(^2\), plasma anticoagulated with citrate is perfused through a membrane oxygenator, a water bath maintained at 37°C and a hollow fibre bioreactor seeded with approximately \(6 \times 10^9\) cryopreserved, dextran microcarrier-attached primary porcine hepatocytes in the extracapillary compartment of the module. This number of cells represents approximately 2% of the normal human liver cell mass. A cellulose-coated charcoal sorbent column is incorporated into the design. Treatment pulses are of relatively short duration of 6–7 h, since loss of hepatocyte viability has been observed after this time\(^3\). In the ‘extracorporeal liver assist device’ (ELAD)\(^4\), blood anticoagulated with heparin is continuously perfused through a hollow fibre bioreactor containing C3A hepatoblastoma cells in attachment culture on the outer surface of the hollow fibre membranes. This module supports the growth of 200 g of cells, representing approximately 20% of human liver cell mass. Continuous treatment for up to 168 h has been reported\(^5\). This design does not incorporate a charcoal column.

In the clinical setting of ALF, each of these devices has been primarily employed as a bridge to OLT. Treatment with the BAL for up to 7 h on 1–3 occasions was associated with reductions in intracranial hypertension and the hepatic encephalopathy index and the survival of
all 12 patients for 21–96 h (mean 39.3 h) until OLT could be performed. Interpretation of the specific influence of the bioreactor per se in this system is precluded by the absence of control groups treated with intensive care or charcoal haemoperfusion alone. Furthermore, results in patients with acute or chronic liver failure were less encouraging, with 0/6 non-OLT candidates surviving. The same group has reported significantly reduced bilirubin, aminotransferase, alkaline phosphatase and ammonia concentrations in peripheral blood following BAL treatment in patients with acute liver failure and significantly reduced bilirubin and ammonia levels in patients with acute on chronic liver failure. In clinical applications reported so far, acute immunological reactions were not observed, even after repeated treatment sessions in the same patient.

Uncontrolled experience with the ELAD has been reported in 11 patients with ALF, who were treated for up to 144 h. One patient recovered without OLT, whilst 4 were maintained until OLT could be performed and the remaining 6 died. The first pilot controlled trial of the use of the ELAD in acute liver failure has recently been reported. In view of the inevitable heterogeneity of the study population with regard to etiology and severity of ALF, patients were stratified into two groups. Group 1 (n = 17) consisted of patients considered on admission to have a 50% chance of recovery, whilst group 2 (n = 7) included patients in whom the chance of spontaneous recovery was considered <5% and who were listed for OLT. Following stratification, patients were then randomly allocated to treatment with ELAD or intensive care alone. Overall, treatment for a median period of 72 h (range 3–168 h) with two ELAD devices connected in series resulted in a significant increase in galactose elimination capacity but no significant change in blood ammonia levels. A reduced incidence of deteriorating encephalopathy grade (3/12, 25% vs 7/12, 58%) was noted in the ELAD-treated group. In group 1, survival in ELAD-treated patients was 7/9 (78%). However, a higher than expected survival in controls was also noted (6/8, 75%). There was no difference in the number of patients who deteriorated to fulfil transplant criteria. In group 2, survival was 1/3 (33%) in ELAD-treated patients and 1/4 (25%) in controls. Both survivors underwent OLT (Fig. 1).

These results highlight the difficulties in designing appropriate clinical studies to assess the efficacy of bioartificial liver support devices in ALF. In the most severe cases requiring OLT, efficacy can only reliably be judged in terms of sustaining patients until this can be performed and is, therefore, liable to the major confounding variable of organ availability. In those with less severe ALF, the use of less stringent inclusion criteria than those employed to list patients for OLT results in a relatively high survival rate in the control group, such that large numbers of patients...
Transplantation

Fig. 1 Survival data from a controlled pilot study of the efficacy of the extracorporeal liver assist device (ELAD), performed in patients stratified into two groups according to likelihood of recovery (reproduced, with permission from the publishers of Hepatology).

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
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<tbody>
<tr>
<td>ELAD</td>
<td>Control</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Progression to transplant criteria (transplanted)</td>
<td></td>
</tr>
<tr>
<td>3 (2)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Survival (%)</td>
<td></td>
</tr>
<tr>
<td>7/9 (78%)</td>
<td>6/8 (75%)</td>
</tr>
</tbody>
</table>

would be required to definitively demonstrate or exclude a treatment benefit. Additional indices of efficacy apart from survival, such as rapidity of recovery, time in hospital and frequency of associated complications, may thus need to be considered when designing future trials. These same considerations would also apply to the design of any trial performed in patients with acute hepatic decompensation on a background of chronic liver disease.

Concluding remarks

Considerable research in recent years has demonstrated the importance of factors such as matrix support and co-culture with non-parenchymal cells for the maintenance of hepatocyte viability and differentiated function in culture, leading to the development of a number of promising bioartificial liver support devices. However, the initial clinical experiences with two of these have indicated that functional efficacy in this setting may be less than in the experimental situation. Several fundamental issues remain unresolved, not the least of which relate to the cell mass required to provide meaningful support and which of those hepatocyte components and bioreactor designs so far proposed is best able to do this. In particular, further studies of the efficacy of devices incorporating human hepatocyte lines transformed by either cultural conditions or genetic engineering and those based on multi-channel or flat bed bioreactor designs in which hepatocytes are co-cultured with non-parenchymal cells are awaited. Whilst testing in animal models of ALF is essential for the initial assessment process, controlled trials on a multicentre basis in well-defined patient groups and with standardised outcome measures will ultimately be required to properly evaluate the
clinical value of these devices. A better understanding of factors promoting recovery and regeneration of the native liver and to what extent these can be provided by extracorporeal devices will be essential to the further development of effective bioartificial liver support systems.

**Key points for clinical practice**

1. Two bioartificial devices have now undergone preliminary clinical evaluation, namely the ‘bioartificial liver’ (BAL) and the ‘extracorporeal liver assist device’ (ELAD), but little controlled data is available and their clinical value is at present unproven.
2. Several other devices with different characteristics have shown promise in vitro and/or in animal models of acute liver failure (ALF) but await clinical evaluation.

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