J Gastroenterol 2000; 35:493–503

Journal of Gastroenterology

Review

Developments in bioartificial liver research: concepts, performance, and applications

Seishi Nagamori[1,3], Satoshi Hasumura[2], Tomokazu Matsuura[2], Hideki Aizaki[3], and Masaaki Kawada[4]

[1] Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, Japan
[2] Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan
[3] National Institute of Infectious Disease, Tokyo, Japan
[4] Department of Public Health and Environment Medicine, Jikei University School of Medicine, Tokyo, Japan

Abstract: As an alternative to liver transplantation, numerous researchers have been working toward the goal of development of a fully functional artificial liver. In recent years, artificial liver support systems have been advocated as interim treatments for patients awaiting hepatocyte replacement therapy or liver transplantation; so-called "bridging" treatments. It is recognized that an effective artificial liver system requires: (1) a viable and highly functional hepatocyte cell line, (2) a suitable bioreactor environment and peripheral control systems, and (3) an effective extracorporeal circulatory system to incorporate an artificial liver system. Conventional systems have, however, suffered from various drawbacks, including incompatibility of cell cultures derived from non-human cells, insufficient cell proliferation, rapid deterioration of cellular function due to an impoverished cellular environment, and lack of system scalability. A newly established artificial liver system overcomes many of these problems and demonstrates a long-term capacity to maintain multiple liver-specific functions, such as protein synthesis, enzyme activity, and drug metabolism, both quantitatively and qualitatively. The present review provides an overview of the concepts underpinning artificial liver systems, the performance of presently available systems and the practical applications of available systems and those in development.

Key words: bioartificial liver, hepatocyte cell line, radial flow bioreactor, bridging, albumin, urea cycle, extracorporeal circulatory system, cytochrome P450

Received: February 7, 2000 / Accepted: February 25, 2000
Reprint requests to: S. Nagamori
Present address: Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Shinkawa, Mitaka, Tokyo 181-8611, Japan

Introduction

Conventional wisdom holds that severe liver failure is most effectively treated using the established techniques of whole- and partial-liver transplantation. A viable bioartificial liver (BAL), on the other hand, would offer superior efficacy while overcoming the practical and ethical issues posed by liver transplantation. The tremendous functional complexity of human liver tissue, however, presents a formidable challenge to the creation of an effective artificial liver: the liver cannot be replicated simply by mechanical devices.[1] Researchers are therefore concentrating their efforts on hybrid systems incorporating human- or animal-derived cells or tissue.[2] Recent advances in the development of inorganic materials having both biocompatibility and high performance have, in fact, made it possible to create such hybrid systems, thus facilitating further research in this area.

While research on BAL systems is still in its infancy,[3,4] the urgent goal is to develop a sophisticated BAL suitable for clinical applications. Researchers in this area, however, have yet to demonstrate sufficient functions of hepatocyte cell lines in culture. Accordingly, we must first ensure that a BAL and its constituent cell culture can be developed and improved to incorporate the multiple functions of normal liver cells. In addition, an advanced BAL should be applicable to areas other than the treatment of severe liver failure, including: the assessment of drug efficacy, analysis of drug metabolism and toxicity, studies of environmental toxins, ex-vivo gene therapy,[5] in-vitro viral research and vaccine development, and the commercial production of many types of liver-specific proteins and physiologically active substances.[6]

While the present review will focus on BAL systems, it should be noted that cell transplantation has shown promising results in animal models of liver failure.[5,7,8] Investigators have successfully overcome immunosup-

pression and tissue rejection through the use of microencapsulation techniques,[9,10] but problems concerning cell viability and regulation of cellular function within the recipient's body remain to be resolved. We believe, however, that sophisticated BAL systems can overcome such problems. The present review will thus attempt to provide an overview of BAL research and development and the status of various systems presently available.

Types of bioartificial liver (BAL) systems

Development of a hybrid BAL

Hybrid systems refer to devices which incorporate living cells or tissues in an inorganic bioreactor vessel.[2] The important point in developing such a system for liver tissue is to ensure that cultured hepatocytes are provided with an environment permitting the expression of the full range of liver functions, including protein synthesis, enzyme activity, bilirubin conjugation, and drug metabolism.[11,12] To reach the desired goal of a fully functional artificial liver system, however, researchers must first succeed in achieving long-term viable culture of human liver-derived cells, as well as succeeding in the development of extracorporeal circulatory systems and highly sensitive control systems.

Ideally, cell cultures exhibiting advanced cellular functions while maintaining long-term viability with consistent proliferation should be derived from freshly isolated human liver parenchymal cells. To this end, parenchymal cells must first be immortalized and allowed to proliferate into stable cell cultures.[13] Unfortunately, while some research teams have prepared cultures based on normal liver parenchymal cells, viable cell cultures displaying proliferative capacity and advanced performance have yet to be achieved. In practice, viable cell cultures have best been obtained by employing human liver cell strains derived and cloned from human hepatoma cells. It is hoped, however, that methods will be developed to allow the use of normal human hepatocytes in long-term, highly functional cultures. The bioreactor vessel is a critical component of any BAL system. Bioreactors can be broadly grouped into two categories:[14–23] (1) stacked hollow fibers wherein cells adhere to the fiber walls in essentially two-dimensional layers; and (2) three-dimensional bioreactors which incorporate suitable substrates enabling cellular adhesion in true three-dimensional clusters. Here, we focus on our development of a compact "radial flow" three-dimensional bioreactor which supports massive, high-density cultures showing long-term viability and superior liver-specific performance.

As mentioned above, the essential requirements for an effective BAL include:

(1) Establishment of a viable and highly functional hepatocyte cell line[24,25]
(2) Development of a suitable bioreactor environment and peripheral control systems
(3) Incorporation within an effective extracorporeal circulatory system.[26,27]

These three aspects of BAL development are combined to create a viable BAL system. Fig. 1 (*A–D*) illustrates

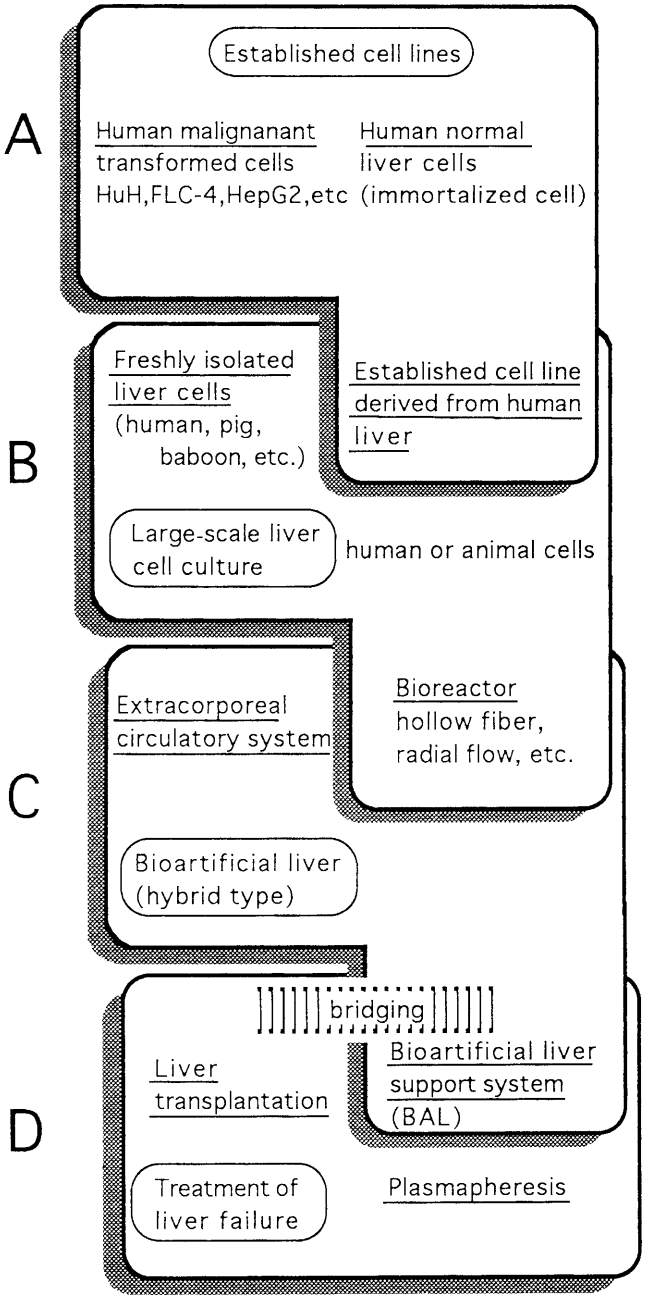

Fig. 1. Bioartificial liver (*BAL*) development process from the establishment of cell lines to comprehensive treatment of liver failure

the processes leading to the successful implementation of a BAL system in the treatment of a patient with liver failure. It is important to examine each of these processes in more detail.

Hepatocytes suitable for BAL systems

The type of hepatocyte upon which a bioreactor culture is developed is a fundamental step with significant implications for the effectiveness of the resulting BAL system. Cell lines can be derived from human or animal liver, or other cells manipulated through genetic modification (Fig. 1*A*, *B*).

Although optimal for BAL applications, the use of human cells is restricted and impeded by complex legal rules which vary by country. Despite these legal obstacles, however, several viable sources can be considered.

(1) Freshly isolated hepatocytes can be obtained from fetal tissue, surgically removed liver, or liver tissue obtained from the bodies of people with brain death. Hepatocytes taken from these sources, however, are very vulnerable to contamination, technically difficult to preserve in a fresh state, and show a sharp decrease in cellular functions after a single culture passage. Although investigators have yet to demonstrate consistent proliferation of human hepatocytes in vitro, the advantages of homology and human-specific functions demand that resources continue to be directed toward the elucidation of the conditions necessary for maintaining viable human hepatocyte cultures.
(2) Cell strains established from human liver cancer cells can be derived from primary hepatocellular carcinoma or hepatoblastoma cells.[25] For example, the well known HepG2 line was derived from hepatoblastoma cells,[28] and 7 cell lines[23,55,77] were derived in our own laboratory from primary hepatocellular carcinoma. Such cells can be employed effectively if the function of normal liver cells has been highly preserved. In practice, however, such cells contain a high proportion of abnormal genetic component, which inhibits their ability to express normal protein synthesis and enzyme activity. Although strenuous efforts are required to establish a stable cell strain using carcinoma cells, such work is rewarded by the ease with which large-scale cell cultures can be generated. Once a strain is established, however, strict vigilance must be applied to prevent viral or substance contamination.
(3) Artificially immortalized human liver cells would be an ideal source if genetic engineering or other methods could be used to imbue the cells with the highly differentiated functions and proliferative capacity observed in normal cells.[13] At present, cultures of such immortalized cells have not demonstrated long-term viability, and the hepatocyte-specific characteristics, such as albumin secretion, disappear relatively quickly. In addition, these cells suffer from a high incidence of malignant change and dedifferentiation. Research continues to be carried out in this field, however, and promising results can be expected in the future.

Animal cells are a more common source. Clinically, BAL systems using such cells have been widely reported in recent years. While the cells are less expensive and readily available in large quantities, the drawbacks include the potential for viral contamination, as well as the physiological dangers and ethical problems posed by xenotransplantation.[24] Furthermore, it has been shown that animal cells undergo malignant transformation relatively easily in vitro, while human cells are more stable.[29] Animal cell cultures generally fall into three categories:

(1) Porcine hepatocytes can easily be preserved in large quantities,[5,30,31] but they present problems of heteroimmunity when used over long periods.[14,20,32]
(2) Simian hepatocytes may be considered favorable as a close proximation of human liver cells, but their use is bound to become more difficult with increasing public awareness of and demand for the protection of animal rights.
(3) Implantation of human hepatic genes into animal cells offers another source for cell cultures. An experiment was conducted in which human hepatic genes were inserted into porcine hepatocytes. Promising results were obtained, giving great expectations for future research in this area.

Another promising line of research has focussed on the coculture of nonparenchymal cells (NPC) with parenchymal cells in a three-dimensional system.[33] Recent work indicates that the differentiation of primary hepatocytes is maintained by coculture with NPC.[34,35] Although the precise roles played by NPC are yet to be determined, it is expected that successful replication of human liver functions will require cocultures of parenchymal and nonparenchymal cells.[36]

BAL-related materials and devices

Advances in the development of BAL systems have largely followed breakthroughs in related technologies. Historically, liver treatment systems proceeded from dialysis membranes, to activated carbon filters, and then to synthetic-resin adsorbents for purifying body fluids.

While detoxification has been a central motivation for these developments,[26,37,38] it is increasingly clear that liver-specific proteins, enzymes, and other factors are necessary for the normal function of the body as a whole. In recurrent hepatic encephalopathy, for example, recovery from coma may be accompanied by the release of a liver-specific substance, which may stimulate relevant neural circuits in the central nervous system. Such a stimulatory substance or factor has not yet been identified, but recent clinical reports note that patients recover from coma after treatment with artificial liver systems.[15,39] Thus, the elucidation of not only detoxification mechanisms but also of synthetic and secretory functions is essential in order to realize the full potential promised by a comprehensive BAL system.[24,37]

The majority of studies of BAL systems have been conducted using two-dimensional culture systems incorporating freshly isolated hepatocytes[20] or cell strains imparted with liver functions. If such cells could be cultured in a three-dimensional environment, the resulting BAL should have higher performance, while achieving very high cell density within a much more compact scale. Ideally, such a device would enable long-term culture of cells expressing features of enhanced hepatic function, including liver-specific protein synthesis, enzyme activity, ammonia metabolism, gluconeogenesis,[22] and activation of drug metabolism.[27] It is useful to compare the differences in such parameters between the two major types of bioreactor in use today.

Most of the reported BAL systems are based on hollow fiber,[20,40] polyvinyl matrix,[22] packed beds,[41] or other systems.[42]

The hollow fiber system, in particular, has enjoyed considerable attention as the most commonly used BAL for clinical application.[20,43] In one study, a patient with hepatic coma was reported to have recovered consciousness after treatment with a hollow fiber system incorporating porcine hepatocytes. Human liver cells cultured in a hollow fiber BAL have also been used clinically to improve the liver functions of a patient with fulminant hepatitis.[37,44] Despite these well reported successes, such systems have several problems in common. Cells affix to the surface of the fibers or beads and aggregate in layers,[41] resulting in severe shear stresses and disruption of the equal distribution of oxygen and nutrients to all cell layers.[27,41,45–47] Moreover, the lack of efficient distribution flow leads to the build-up of waste products and debilitating fluctuations in pH levels and gas concentrations. Accordingly, productivity and function are reduced, and the life span of the cell culture is shortened. These issues all present major obstacles to the successful implementation of such systems for comprehensive, long-term treatment.

The three-dimensional bioreactor

Three-dimensional culture differs from other system fundamentally. The representative structure comprises a radial flow bioreactor (RFB) column, such as that illustrated in Fig. 2.[48–51] The RFB column consists of a vertically extended cylindrical matrix comprised of porous glass bead microcarriers, through which liquid medium flows from the periphery toward the central axis, generating a beneficial concentration gradient of oxygen and nutrients, while preventing excessive shear

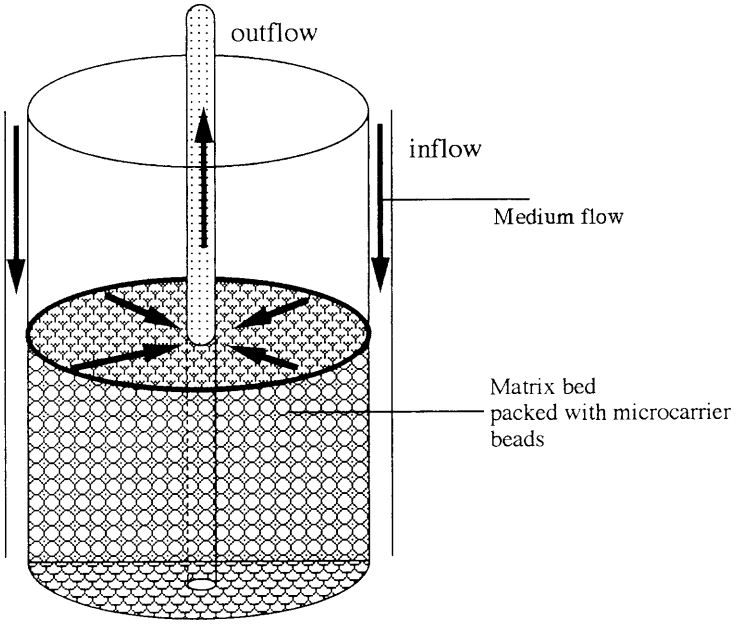

Fig. 2. Radial flow bioreactor column. The medium flows radially through a matrix bed packed with microcarrier beads, resulting in an even distribution of nutrients and elimination of waste without excessive shear stress

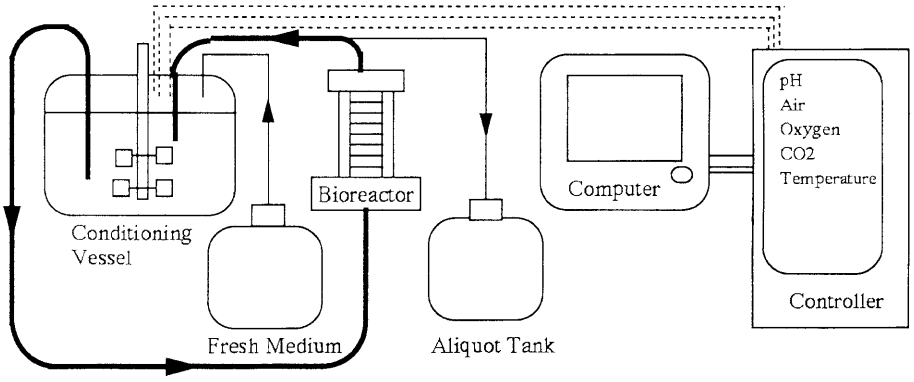

Fig. 3. Radial flow bioreactor system. The bioreactor system is placed in an aseptic room maintained at 37°C. The system is composed of a radial flow bioreactor and a conditioning vessel, which is connected to a circulation system including a fresh medium supply tank and recovery aliquot tank. The system is automatically controlled by a computer system monitoring pH values; glucose, oxygen, and CO_2 consumption; and temperature

stresses or build-up of waste products.[23] Sophisticated peripheral devices supplying and monitoring nutrients, oxygen and pH, among other parameters, are automatically controlled by computer (Fig. 3). The highly porous microcarrier bead structure provides a vast surface area for the adhesion and proliferation of cell colonies in three dimensions, and the system supports high-density, large-scale cultures having long-term viability.[16,42,46,52] Recent studies have revealed that the cells' natal morphology and function can be maintained and conditions closely resembling the in-vivo state can be achieved.[23] A further research study has demonstrated that the system supports high-density, large-scale cell cultures having a long-term capacity to maintain multiple liver-specific functions, such as protein synthesis, enzyme activity, and drug metabolism, both quantitatively and qualitatively.[53]

In summary, the RFB system has overcome the following problems associated with conventional BAL reactors:[49–51,54]

(1) Influence of shear stress
(2) Uneven supply of oxygen and nutrients
(3) Build-up of waste substances
(4) Gravitational sedimentation of cells
(5) Incomplete three-dimensional culture
(6) Poor cost-to-performance ratio
(7) Difficulty to scale up or scale down.

Employing this RFB system together with the FLC cell lines[55] developed in our laboratory, we have confirmed rapid and prolonged proliferation of FLC cell cultures showing normal levels of oxygen and glucose consumption, as well as albumin synthesis and ammonia metabolism. Cellular consumption of oxygen and glucose increases from the start of cultivation and has repeatedly been maintained throughout cultivation terms lasting more than 3 months (see Fig. 4).

Cellular secretion of the liver-specific proteins albumin and alpha-fetoprotein (AFP) has also been demonstrated in both monolayer and three-dimensional

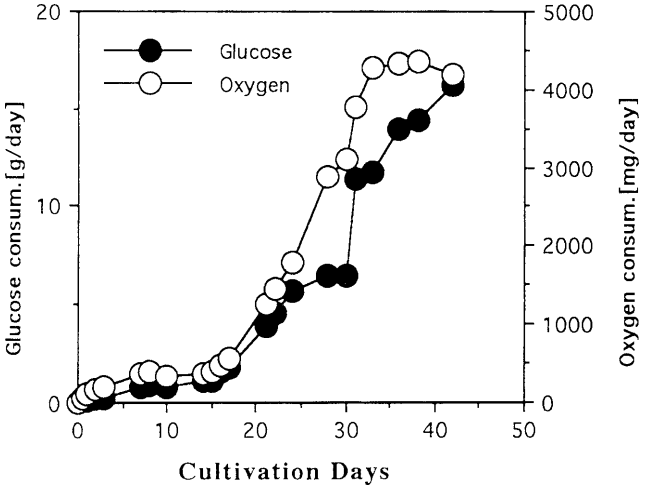

Fig. 4. Glucose and oxygen consumption (*consum.*) rates. FLC-4 cells cultured in a radial flow bioreactor show normal levels of oxygen and glucose consumption throughout the 40-day culture period

RFB cultures, but the relative quantities produced showed dramatic differences depending on the culture method. As illustrated in Fig. 5, monolayer cultures produced greater quantities of AFP than albumin, while RFB cultures showed the reverse results. In RFB culture, albumin was synthesized in quantities of more than 12 g per day, sevenfold greater than that observed in monolayer cultures, while alpha-fetoprotein decreased to 1/400th of the monolayer levels. These results indicate that the RFB system was conducive to differentiation of function in the FLC cells.

As shown in Fig. 6, the addition of 1 mM ammonia to an RFB culture led to a rise in ammonia level, but there was no alteration in urea concentration. The subsequent addition of 3 mM ammonia induced an immediate and prolonged rise in urea concentration, followed by a corresponding drop in ammonia levels through to the end of the 40-day cultivation term.

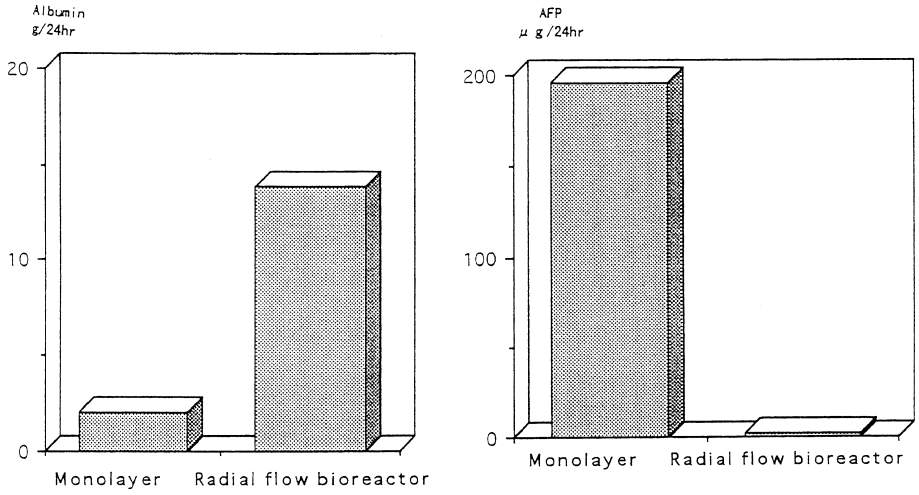

Fig. 5. Comparison of secreted albumin and alpha fetoprotein (*AFP*). FLC cells secrete much greater quantities of albumin than AFP when cultured in a radial flow bioreactor, but the opposite result is obtained in monolayer culture

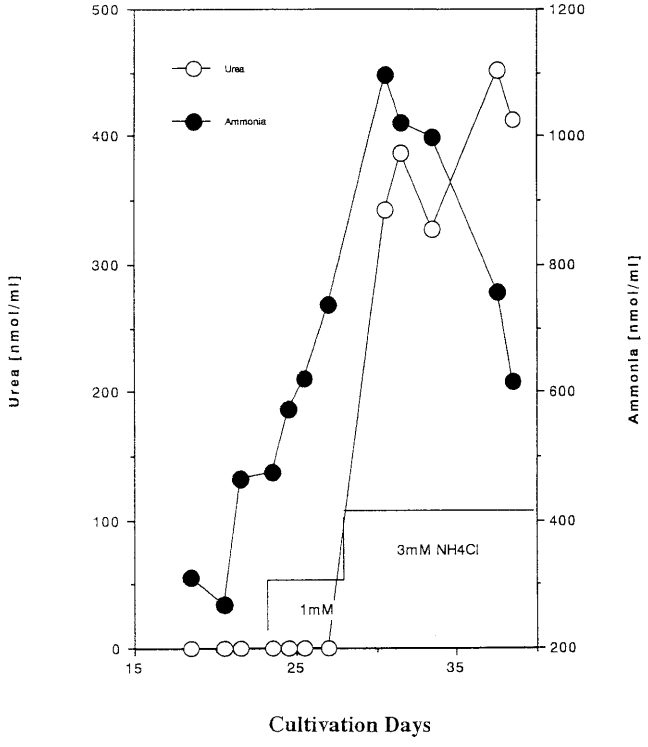

Fig. 6. Urea synthesis and ammonia concentration. The addition of 1 mM ammonia to a bioreactor culture of FLC cells leads to a rise in ammonia levels, but there is no alteration in urea concentrations. The subsequent addition of 3 mM ammonia leads to an immediate and prolonged rise in urea concentrations, followed by a corresponding drop in ammonia levels through to the end of the 40-day cultivation term

As indicated by the above findings, we have been able to confirm normal levels of liver-specific functions in a series of experiments. Studies presently underway in our laboratory have built on these data and the findings are reported later in this review.

Extracorporeal BAL system[26,27]

In considering the development of BAL systems, we must look at the incorporation of the BAL reactor within a suitable extracorporeal circulation system (Fig. 1*C* and Fig. 7). Advanced computer-control systems have greatly contributed to the improved performance of BAL devices. In clinical extracorporeal applications, the RFB is combined with a conventional plasma separator[56] and other relevant devices.[1] A cell filter is incorporated to prevent cultured cells from entering the circulatory system. The anti-coagulant, nafamostat mesilate, is employed to prevent blood coagulation. To maintain the smooth operation of the combined systems, computer controls are applied to precisely monitor and regulate temperature, circulation flow rate, cell density, pH, filter pressures, the concentration of the nutrient medium, and oxygen supply on a continuous basis.

Applications of BAL

Hepatic failure

Recently developed BAL systems have increasingly found clinical applications.[57] At present, systems have primarily been applied as a "bridging" mechanism in patients with hepatic failure (Fig. 1*D*). In the terminal stages of hepatic failure, the management of cholestasis is a critical problem. Conventional devices using adsorbents such as charcoal can be attached to the BAL to eliminate bilirubin[11,12] and endotoxins, thus providing the basic functions required for clinical applications. Such methods can provide life-support for patients with liver failure and can improve the patient's general condition while a suitable donor organ for liver transplantation is found.

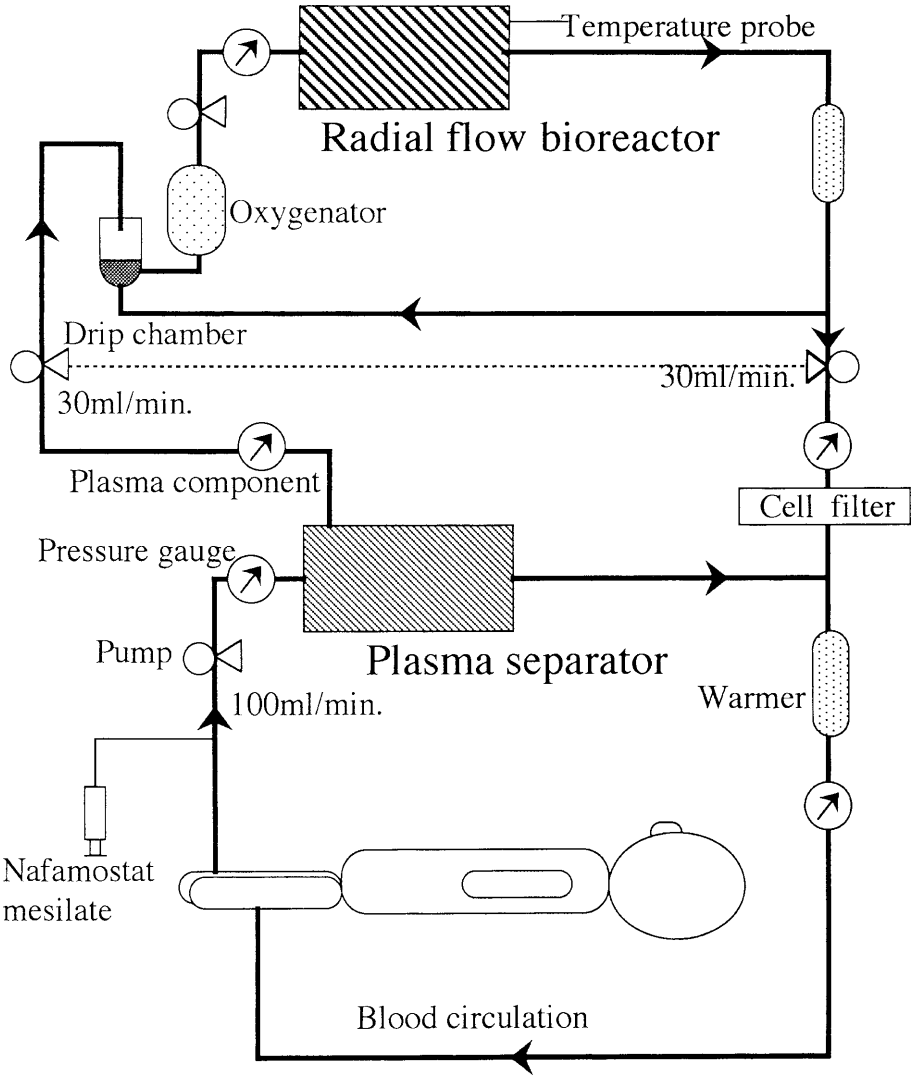

Fig. 7. Extracorporeal BAL device

In a recently reported clinical study carried out in the United States, a hollow fiber bioreactor charged with porcine hepatocytes was used to treat patients with fulminant hepatitis or chronic liver disease. All patients received treatments for plasma exchange, blood dialysis, and filtration of adsorbed plasma.[26,56] The hollow fiber bioreactor was reported to be effective in providing a so-called "bridge"[58,59] to ameliorate symptoms such as disturbance of consciousness or brain edema in patients with fulminant hepatitis.[20,39,60,61] Some of the patients showed recovery after auxiliary BAL treatment alone. Similarly, some patients with chronic hepatitis also showed recovery during acute phases of the disease.

These findings are indeed remarkable and point to an optimistic future for further research, especially given that the volume of porcine hepatocytes per charge was only a small fraction of that found in a normal human liver. It would, however, be beneficial to pursue studies based on a more rigorous and controlled evaluation which includes objective diagnostic criteria in both laboratory and clinical settings.[62,63]

In a separate study, patients with hepatic encephalopathy were reported to have recovered from coma in response to BAL treatment. Here, it is important to stress that BAL treatment is markedly different from conventional methods in that it not only eliminates toxins but also produces many critical substances that, would normally be synthesized by liver parenchymal, endothelial, and other specialized cells.

Drug detection and toxicology

Animals such as the rat, dog, or pig have conventionally been used to test the toxicity or carcinogenicity of various chemicals, as well as their kinetics in the body.[14,64–66] It is well known, however, that marked differences exist between humans and other animals in

regard to drug metabolism and excretion. It is therefore essential to consider such differences when experimental data are being evaluated for clinical applications. Furthermore, increased awareness of and support for animal welfare in the general population requires investigators to rely less on animal subjects and instead develop suitable experimental models using humans or human tissue systems. Attention is thus being directed to recent developments in the use of BAL systems incorporating human hepatocyte-derived cells capable of liver-specific functions, in combination with suitable culture environments that replicate the three-dimensional, high-density tissue structures present in normal human liver. Indeed, ongoing research appears to indicate that suitable three-dimensional bioreactor systems can provide meaningful data for drug metabolism[27] and toxicology.

As illustrated in Figs. 5 and 6, a high-density three-dimensional RFB culture can synthesize normal levels of albumin and can eliminate toxic substances such as ammonia.[22] Such findings are not readily observed in two-dimensional culture systems. In our laboratory, recent studies of cytochrome P450 (CYP;[67,68]), an oxidase which acts to regulate the metabolism of toxic substances, have revealed that human liver-derived cell strains display human-specific CYP isozyme and carboxylesterase activity. Figure 8 shows the response of FLC 4 cells to omeprazol challenge. The data indicate

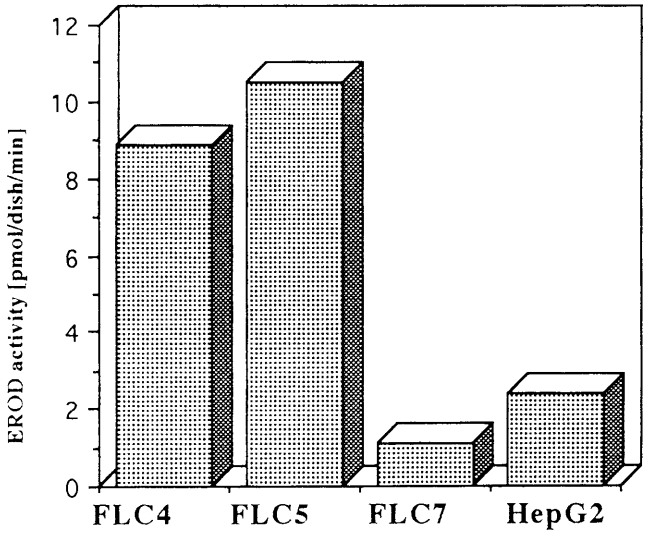

Fig. 8. Effect of omeprazol on 7-ethoxyresorfin o-deethylase (*EROD*) activity in FLC and HepG2 cell lines. The fig. shows EROD activity of FLC cells in response to omeprazol, a known inducer of cytochrome isozyme CYP1A. CYP1A activity in both the FLC 4 and FLC 5 cell lines was found to be robust, and was significantly greater than that in HepG2 cell lines (data obtained from studies conducted in collaboration with Professor K. Chiba of Chiba University, Japan)

that the FLC cells are capable of metabolizing drugs via human-specific metabolic enzymes in vitro.[69]

Ongoing research in our group suggests that the RFB system could be used in place of animals as a drug-detection and toxicology model which more closely replicates human liver functions. This type of in-vitro system is expected to facilitate future research in terms of the following applications:

(1) Analysis of drug metabolism in the liver
(2) Studies of drug interaction
(3) Detection of drug-derived mutagens in the liver
(4) Toxicology of environmental pollutants.

Drug production and synthesis

An important area of study that has both commercial and medical implications concerns the application of BAL systems to drug production and protein synthesis. As we showed in Fig. 5, the RFB system demonstrated robust synthesis of albumin protein at normal levels. Furthermore, recent unpublished results from our laboratory reveal that the same system can produce human thrombopoietin (TPO) at normal levels, and others have shown that cultured cells release several prostaglandin species in response to acute cellular inflammation.[70] It is important to realize that the RFB is made up of small, repeatable components that can be expanded or contracted in size to rapidly scale the system up or down. We could thus scale up a human-hepatocyte bioreactor culture to obtain large-volume production of useful physiological substances. Drugs, proteins, or other agents produced in this way will be less infiltrated by foreign contaminants than these items produced by genetic engineering, or by using yeast, *Escherichia coli*, or cloned animals, and can be easily separated from other components to obtain the target substance.

Human hepatitis infection model

It is acknowledged that the recent explosion of molecular and genetic based research on viral agents and therapies is impeded by the lack of suitable experimental models of in-vitro cellular infection. Specifically, while knowledge of the hepatitis C virus (HCV) is rapidly expanding, it is largely confined to studies at the genetic level, while confirmation of the virus itself in living tissue has been elusive, because in-vitro culture of hepatocytes has yet to be established.[71] This lack of a suitable HCV infection model is not only holding back basic studies on viral biology and carcinogenicity, as well as viral particulate formation and mutation, but is inhibiting the development of preventive vaccines and drugs such as protease inhibitors and antisense blockers.

At present, there is no alternative to the chimpanzee model. It is an expensive animal, and differs from humans in subtle but possibly crucial respects. Studies based on chimpanzee models commonly suffer from great variance in experimental data. Furthermore, the influence of animal rights movements is restricting the availability of such animals for use in research. Numerous investigators are thus calling for the establishment of an experimental model based on suitable in-vitro cell culture systems, independent of clinical studies or animal research.[71,72,73,74]

In a groundbreaking series of studies, our group[75,76] has succeeded in identifying HCV RNA protein,[77] replication, and core viral proteins at the picogram level in a long-term RFB culture. Further work is presently being conducted in an effort to establish an experimental system for the in-vivo inoculation and detection of HCV infection, as well as for the clarification of infection mechanisms and viral proliferation, and the testing of potential therapies and preventive measures.

Conclusion

Developments in BAL technologies and their applications in clinical settings suggest that fully functional BALs could be an essential part of liver therapy in the near future. In particular, the new findings obtained in the-three dimensional RFB system support the view that the system has great potential as an effective artificial liver support system, and as a vehicle for research on drug metabolism and environmental toxicity, ex-vivo gene therapy, and for the commercial production of many types of liver-specific proteins and cytokines[70] for therapeutic use. Perhaps most importantly, such a system, in combination with appropriate cell cocultures, offers us the ability to investigate the factors that promote the recovery and regeneration of native liver and contribute to toward a comprehensive understanding of the human liver and its maintenance and treatment.

Acknowledgments. We would like to thank Dr. S. Mizutani, of the Institute of the Kirin Brewery Co Ltd; Drs. K. Chiba and M. Hosokawa of the Department of Pharmacology and Toxicology, Chiba University; and Drs. T. Miyamura Y. Matsuura of the National Institute of Diseases for their kindly assistance and advice.

This work was supported in part by a Grant-in-Aid of the Japanese Ministry of Education, Science and Culture, the Japan Health Sciences Foundation, and the Research on Health Sciences focusing on Drug Innovation.

References

1. Catapano G. Mass transfer limitations to the performance of membrane bioartificial liver support devices. Int J Artif Organs 1996;19:18–35.
2. Rozga J, Holzman MD, Ro M-S, Griffin DW, Neuzil DF, Giorgio T, et al. Development of a hybrid bioartificial liver. Artif Organs 1993;217:502–11.
3. Cattral MS, Levy GA. Artificial liver support—pipe dream or reality? New Engl J Med 1994;331:268–9.
4. Kasai S, Sawa M, Mito M. Is the biological artificial liver clinically applicable? A historic review of biological artificial liver support systems. Artif Organs 1994;18:348–54.
5. Kay MA, Faust N. Liver regeneration: prospects for therapy based on new technologies (review). Mol Med Today 1997;3:108–15.
6. Selden C, Shariat A, Mcloskey P, Ryder T, Roberts E, Hodgson H. Three-dimensional in vitro cell culture leads to a marked upregulation of cell function in human hepatocyte cell lines—an important tool for the development of a bioartificial liver machine. Ann NY Acad Sci 1999:353–63.
7. Dixit V, Gitnick G. Transplantation of microencapsulated hepatocytes for liver function replacement. J Biomater Sci Polymer Edn 1995;7:343–57.
8. Nagaki M, Kano T, Muto Y, Yamada T, Ohnishi H, Morikawa H. Effects of intraperitoneal transplantation of microcarrier-attached hepatocytes on D-galactosamine-induced acute liver failure in rats. J Gastroenterol 1990;25:78–87.
9. Dixit V. Transplantation of isolated hepatocytes and their role in extrahepatic life support systems. Scand J Gastroenterol 1995;30(Suppl 208):101–10.
10. Stange J, Mitzner S, Dauzenberg H, Ramlow W, Knippel M, Steiner M, et al. Prolonged biochemical and morphological stability of encapsulated liver cells. A new method. Biomater Artif Cells Immobilization Biotechnol 1993;21:343–52.
11. Demetriou AA, Levenson SM, Novikoff PM, Novikoff AB, Chowdhury NR, Whiting J, et al. Survival, organization, and microcarrier-attached hepatocytes transplanted in rats. Proc Natl Acad Sci USA 1986;83:7475–9.
12. Fremond B, Malandain C, Guyomard C, Chesne C, Guilouzo A, Campion J-P. Correction of bilirubin conjugation in the gunn rat using hepatocytes immobilized in alginate gel beads as an extracorporeal bioartificial liver. Cell Transplant 1993;2:453–60.
13. Werner A, Duvar S, Muentemeyer J, Kahmann U, Luensdorf H, Lehmann J. Cultivation and characterization of a new immortalized human hepatocyte cell line, HepZ, for use in an artificial liver support system. Ann NY Acad Sci 1999;875:364–8.
14. Baquerizo A, Mhoyan A, Kearns-Jonker K, Arnaout AS, Shackleton C, Busuttil RW, et al. Characterization of human xenoreactive antibodies in liver failure patients exposed to pig hepatocytes after bioartificial liver treatment. Transplantation 1999;67:5–18.
15. Nyberg SL, Shatford RA, Peshwa MV, White JG, Cerra FB, Hu WS. Evaluation of a hepatocyte-entrapment hollow fiber bioreactor: a potential bioartificial liver. Biotech Bioeng 1993; 41:194–203.
16. Sielaff TD, Yu MY, Amiot B, Rollins MD, Rao S, MacGuire B, et al. Gel-entrapment bioreactor liver therapy in galactosamine hepatitis. J Surg Res 1995;59:179–84.
17. Shatford RA, Nyberg SL, Meier SJ, White JG, Payne WD, HulF W-S, et al. Hepatocyte function in a hollow fiber bioreactor: a potential bioartificial liver. J Surg Res 1992;53:549–57.
18. Sussman NL, Chong MG, Koussayer T, He D-E, Shang TA, Whisenhand HH, et al. Reversal of fulminant hepatic failure using an extracorporeal liver assist device. Hepatology 1992;16:60–5.
19. Takeshita K, Ishibashi H, Suzuki M, Yamamoto T, Akaike T, Kodama M. High cell density culture system of hepatocytes entrapped in a three-dimensional hollow fiber module with collagen gel. Artif Organs 1995;19:191–3.
20. LePage EB, Rozga J, Rosenthal JP, Watanabe F, Scott HC, Talke AM, et al. A bioartificial liver used as a bridge to liver transplantation in a 10-year-old boy. Am J Crit Care 1994;3:224–7.

21. Dixit V. Development of a bioartificial liver using isolated hepatocytes. Artif Organs 1994;18:371–84.
22. Sato Y, Ochiya T, Yasuda Y, Matsubara K. A new three-dimentional culture system for hepatocytes using reticulated polyurethane. Hepatology 1994;19:1023–8.
23. Kawada M, Nagamori S, Aizaki H, Fukaya K, Niiya M, Matsuura T. Massive culture of human liver cancer cells in a newly developed radial flow bioreactor system: ultrafine structure of functionally enhanced cell lines. In Vitro Cell Dev Biol 1998;34:109–15.
24. Stange J, Mitzner S. Cell sources for bioartificial liver support (review). Int J Artif Organs 1996;19:14–7.
25. Nakabayashi H, Taketa K, Miyano K, Yamane T, Sato J. Growth of human hepatoma cell lines with differentiated functions in chemically defined medium, Cancer Res 1982;42:3858–63.
26. McLaughlin BE, Tosone CM, Custer LM, Mullon C. Overview of extracorporeal liver support system—clinical results. Ann NY Acad Sci 1999;875:310–25.
27. Nyberg SL, Shirabe K, Peshwa MV, Shielaff TD, Crotty PL, Mann HJ, et al. Extracorporeal application of a gel-entrapment, bioartificial liver: demonstration of drug metabolism and other biochemical functions. Cell Transplant 1993;2:441–52.
28. Aden DP, Fogel A, Plotkin S, Damjnov I, Knowles BB. Controlled synthesis of HBsAg in differentiated human liver carcinoma-derived cell line. Nature 1979;282:615–6.
29. Kuroki T, Huh N. Why are human cells resistant to malignant cell transformation in vitro? Jpn J Cancer Res 1993;84:1091–100.
30. Koebe HG, Schidberg FW. Isolation of porcine hepatocytes from slaughterhouse organs. Int J Artif Organs 1996;19:53–60.
31. Koebe HG, Paehrnik SA, Sproede M, Thasler WE, Schidberg FW. Porcine hepatocytes from slaughterhouse organs. An unlimited resource for bioartificial liver devices. ASAIO J 1995;41:189–93.
32. Takahashi M, Ishikawa H, Takahashi C, Nakajima Y, Matsushita M, Matsue H, et al. Immunologic considerations in the use of cultured porcine hepatocytes as a hybrid artificial liver. Anti-porcine hepatocyte human serum. ASAIO J 1993;39:M242–6.
33. Bader A, Knop E, Boeker K, Fruehauf N, Schuettler W, Oldhafer K. A novel design for in vitro reconstruction of in vivo liver characteristics. Artif Organs 1995;19:368–74.
34. Busse B, Gerlach JC. Bioreactors for hybrid liver support: historical aspects and novel designs. Ann NY Acad Sci 1999;875: 326–39.
35. Okamoto M, Ishida Y, Koegh A, Strain A. Evaluation of the function of primary human hepatocytes co-cultured with the human hepatic stellate cell (HSC) line LI90. Int J Artif Organs 1998;21:353–9.
36. Strain AS. Ex vivo liver cell morphogenesis: one step nearer to the bioartificial liver? Hepatology 1999;29:288–9.
37. Watanabe FD, Arnalout WS, Ting P, Navvaro A, Khalili T, Kamohara Y, et al. Artificial liver. Transplant Proc 1999;31:371–3.
38. Stange J, Mitzner SR, Risler T, Erley CM, Lauchart W, Goehl H. Molecular adsorbent recycling system (MARS): clinical results of a new membrane-based blood purification system for bioartificial liver support. Artif Organs 1999;23:319–30.
39. Demetriou AA, Rozga J, Podesta L, Lepage E, Morsiani E, Moscioni AD, et al. Early clinical experience with a hybrid bioartificial liver. Scand J Gastroenterol 1995;30(Suppl 208):111–7.
40. Jauregui HO, Mullon CJP, Trennker D, Naika S, Santangini H, Press P, et al. In vivo evaluation of a hollow fiber liver assist device. Hepatology 1995;21:460–9.
41. Li AP, Barker G, Beck D, Colburn S, Monsell R, Pellgrin C. Culturing of primary hepatocytes as entrapped aggregates in a packed bed bioreactor. A potential bioartificial liver. In Vitro Cell Dev Biol 1993;29A:249–54.
42. Hubbel JA, Langer R. Special report: tissue engineering. Washington: American Chemical Society; 1995. p. 42–54.
43. Baquerizo A, Mhoyan A, Shirwan H, Swensson J, Busuttil RW, Demetriou AA, et al. Xenoantibody response of patients with severe acute liver failure exposed to porcine antigens following treatment with a bioartificial liver. Tranplant Proc 1997;29: 964–5.
44. Sussman NL, Kelly JH. Improvement in liver function following treatment with an extracorporeal liver device. ASAIO Trans 1993;17:27–30.
45. Smith MD, Smithwaite AD, Cairns DE, Cousins RB, Gaylor JD. Techniques for measurement of oxygen consumption rates of hepatocytes during attachment and post-attachment. Int J Artif Organs 1996;19:36–44.
46. Foy BD, Lee J, Morgan J, Toner M, Tompkins RG, Yarmush ML. Opitimization of hepatocyte attachment to microcarriers: importance of oxygen. Biotech Bioeng 1992;42:579–88.
47. Nishikawa M, Uchino J, Matsushita M, Takahashi M, Taguchi M, Koike M, et al. Optimal oxygen tension conditions for functioning cultured hepatocytes in vitro. Artif Organs 1996;20:169–77.
48. Matsuura T, Kawada M, Hasumura S, Nagamori S, Obata T, Yamaguchi M. High density culture of immortalized liver endothelial cells in the radial-flow bioreactor in the development of an artificial liver. Int J Artif Organs 1998;21:229–34.
49. Yoshida H, Mizutani S, Ikenaga H. Scale-up of interleukin-6 production by BHK cells using a radial-flow reactor packed with porous glass beads. J Ferment Bioeng 1997;84:279–81.
50. Yoshida H, Mizutani S, Ikenaga H. Continuous production of erythropoietin using a radial flow bioreactor. In: Sasaki R, Ikura K, editors. Animal cell culture and production of biologicals. Dordrecht: Kluwer Academic; 1990. p. 329–34.
51. Yoshida H, Mizutani S, Ikenaga H. Production of monoclonal antibodies with a radial flow bioreactor. In: Kaminogawa S, Ametani A, Hachimura S, editors. Animal cell technology: basic and applied aspects. Dordrecht: Kluwer Academic; 1992. p. 347–53.
52. Gerlach JC, Kloeppel K, Mueller C, Schnoy N, Smith MD, Neuhaus P. Hepatocyte aggregate culture technique for bioreactors in hybrid liver support systems. Int J Artif Organs 1993;16:843–6.
53. Hasumura S, Matsuura T, Aizaki H, Kawada M, Mizutani S, Nagamori S. A fine performance of albumin production of human liver cancer cell lines using a radial flow bioreactor (in Japanese with English abstract). Artificial Blood 1997;5:33–7.
54. Ledezma GA, Folch A, Bhatia SN, Balis UJ, Yarmush ML, Toner M. Numerical model of fluid flow and oxygen transport in a radial-flow microchannel containing hepatocytes. J Biomech Eng 1999; 121:58–64.
55. Nagamori S, Fujise K, Homma S, Sujino H, Matsuura T, Shimizu K, et al. Protein secretion of human cultured liver cells (in Japanese with English abstract). Human Cell 1988;1:382–90.
56. LePage EB, Lane R, McKay D, Rozga J, Demetriou AA. Plasma separation for liver support. J Clin Apheresis 1995;10:70–5.
57. Watanabe FD, Shackleton CR, Cohen SM, Goldman DE, Arnaout WS, Hewitt W, et al. Treatment of acetaminophen-induced fulminant hepatic failure with a bioreactor. Transplant Proc 1997;29:487–8.
58. Watanabe FD, Mullon CJ, Hewitt WR, Arkadopoulos N, Kahaku E, Eguchi S, et al. Clinical experience with a bioartificial liver in the treatment of severe liver failure. A phase I clinical trial. Ann Surg 1997;225:484–94.
59. Neuzil DF, Rozga J, Moscioni AD, Ro M-S, Hakim R, Arnaout WS, et al. Use of novel bioartificial liver in patient with acute liver insufficiency. Surgery 1993;113:340–3.
60. Holstege A, Lock G, Koellinger M, Schoelmrich J. Conservative treatment of acute hepatic failure. Z Gastroenterol 1996;34:192–201.
61. Lozga J, Podesta L, LePage E, Hoffman S, Morsiani E, Sher L, et al. Control of cerebral oedema by total hepatectomy and extracorporeal liver support in fulminant hepatic failure. Lancet 1993;342:898–9.

62. Koebe HG, Schildberg FW. The artificial liver—an interim report. Wien Klin Wochenschr 1998;110:551–63.
63. Thasler WE, Koebe HG, Pahernik SA, Schirdberg FW. Artificial liver on its way to clinical reality? (in German with English abstract). Zentralbl Chir 1995;120:614–23.
64. Velde AAt, Ladiges NCJJ, Flendrig LM, Chamlueau RAFM. Functional activity of isolated pig hepatocytes attached to different extracellular matrix substrates. Implication for application of pig hepatocytes in a bioartificial liver. J Hepatol 1995;23:184–92.
65. Kasai S, Mito M. Large-scale cryopreservation of isolated dog hepatocytes. Cryobiology 1993;30:1–11.
66. Gerlach J, Joerres A, Trost O, Hole O, Vienken J, Courtney JM, et al. Side effects of hybrid liver support therapy: TNF-α liberation in pigs, associated with extracorporeal bioreactors. Int J Artif Organs 1993;16:604–8.
67. Hu MY, Cipolle M, Sielaff T, Lovdahl MJ, Mann HJ, Remmel RP, et al. Effects of hepatocyte growth factor on viability and biotransformation functions of hepatocytes in gel entrapped and monolayer culture. Crit Care Med 1995;23:1237–42.
68. Nyberg SL, Mann HJ, Remmel RP, Hu W-S, Cerra FB. Pharmacokinetic analysis vertfies P450 function during in vitro and in vivo application of a bioartificial liver. ASAIO J 1993;39: M252–6.
69. Shimizu H. Experimental models of liver specific metabolism of environmental pollutants using human derived liver cells in a radial flow bioreactor. Japanese Ministry of Education, Basic science research (A2) report no. 08557033 (in Japanese and English). Tokyo: Japanese Ministry of Education, 1998.
70. Hughes RD, Nicolaou N, Langley PG, Ellis AJ, Wendon JA, Williams R. Plasma cytokine levels and coagulation and complement activation during use of the extracorporeal liver assist device in acute liver failure. Artif Organs 1998;22:854–8.
71. Cohen J. The scientific challenge of hepatitis C. Science 1999;285: 26–30.
72. Shimizu YK, Igarashi H, Kiyohara T, Shapiro M, Wong DC, Purcell RH, et al. Infection of chimpanzee with hepatitis C virus grown in cell culture. J Gen Virol 1998;79:1383–6.
73. Bassett SE, Thomas DL, Brasky KM, Lanford RE. Viral persistence, antibody to E1 and E2, and hypervariable region 1 sequence stability in hepatitis C virus-inoculated chimpanzees. J Virol 1999;73:1118–26.
74. Ito T, Mukaigawa J, Zuo J, Hirabayashi Y, Mitamura K, Yasui K. Cultivation of hepatitis C virus in primary hepatocytes culture from patients with chronic hepatitis C hepatitis results in release of high titre infectious virus. J Gen Virol 1996;77:1943–54.
75. Aizaki H, Nagamori S, Aoki Y, Ishii K, Suzuki T, Matsuura Y, et al. The utilization of human hepatocyte in the study of hepatitis C virus; establishment of the efficient replication system of hepatitis C virus (in Japanese with English abstract). Tiss Cult Res Commun 1999;18:265–78.
76. Aoki Y, Aizaki H, Shimoike T, Tani H, Ishii K, Saito I. A human liver cell line exhibits efficient translation of HCV RNAs produced by a recombinant adenovirus expressing T7 RNA polymerase. Virology 1998;250:140–50.
77. Lohmann V, Korner F, Koch J, Herian U, Theilmann L, Bartenschlager R. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 1999;285:110–3.