REVIEW ARTICLE

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Bioartificial Liver, an Extra Corporeal Hepatic Support: Current Perspectives

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The liver is a vital and remarkably complex organ with array of functions that have effects on nearly every other system of the human body. Transplantation, the only effective means of treating liver failure is not an option for many patients due to its massive cost, invasiveness and risk associated with transplantation. Ironically, liver is a highly regenerative organ. Hence some patients currently undergoing a liver transplant will need not undergo this major surgery if there were a simpler means of obtaining liver functions until their own organ has recovered. In light of the increasing incidence of liver disease and continuing shortage of donor organs, cell-based therapies are gaining attention as promising treatments for liver failure. However the impetus for developing a bioartificial liver is to serve as a bridge to liver transplantation in patients with acute liver failure. The use of bioartificial liver improves the patient's conditions and allows recovery from some complications of chronic liver disease prior to transplantation, which might well prove, both medically effective and economical.

The liver is the largest organ in the body and it is involved in a wide array of functions. The liver is the principle site of synthesis of all circulating proteins apart from γ-globulins. The liver also synthesizes all factors involved in coagulation (apart from factor VIII)–that is, fibrinogen, prothrombin, factors V, VII, IX and XIII, protein C and S and antithrombin. Transport or carrier proteins such as transferrin and caeruloplasmin, acute-phase proteins and other proteins are also produced in the liver. Glucose homeostasis and the maintenance of the blood sugar is a major function of the liver. The liver also has a major role in the metabolism of lipoproteins. The liver catabolizes hormones such as insulin, glucagon, oestrogens, growth hormone, glucocorticoids and parathyroid hormone. It is also the prime target organ for many hormones. It is the major site for the metabolism of drugs and alcohol. The reticuloendothelial system of the liver contains many immunologically active cells. The liver acts as a sieve for the bacterial and other antigens carried to it via the portal tract from the gastrointestinal tract. The antigens are phagocytosed and degraded by Kupffer cells, which are macrophages attached to the endothelium[1-6]. The liver regulates the conversion of toxic ammonia to less toxic urea for filtration and excretion by the kidneys[5-8]. The liver performs all the process of secretion, regulation and storage via the use of specific genes for operation that are located on the chromosomes inside the nucleus of the hepatocytes[9-13]. Since the liver is such an essential organ in the human body, if damaged, one's survival would be in danger.

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LIVER FAILURE

Primary liver diseases are the most frequent diseases worldwide. The American Liver Foundation reports that liver failure is the seventh leading cause of death in the United States and it is estimated that 10 million people have some form of liver disease or impairment that results from infection, cirrhosis, drug overdose, chemical toxicity and other causes. When this process occurs in healthy individuals with normal livers, it is termed as acute liver failure (ALF)[14]. Loss of liver function that complicates chronic liver diseases is termed as acute-on-chronic liver failure[14]. ALF remains a devastating illness with over 60% mortality with conventional

treatment[15]. Liver failure may also occur due to viral hepatitis.

The key feature in most acute or chronic liver disease is alteration of hepatocytes progressing to necrosis[16]. The hepatocytic alterations are the morphologic basis for the main clinical manifestations and for the aberrations in the hepatic test results in acute and frequently in chronic liver diseases. Lesions of hepatocytes are conveniently recognized as (a) alterations of hepatocytic nuclei and of cytoplasm (such as clumping); (b) as excess of physiologic substances such as fat, water pigment and particularly bile; (c) deposition of substances not seen in normal hepatocytes, such as hyaline of Mallory and globules of a - antitrypsin; and (d) hepatocytic necrosis if varying extends with loss of single hepatocytes, best appreciated by the accompanying inflammation[16]. In severe and potentially fatal liver diseases, necrosis and circulatory insufficiency are the reasons for hepatic failure.

The consequences of hepatic injury depend on the efficiency of regeneration in restoring or replacing the lost hepatocytic function[16]. Regeneration of hepatocytes assumes a nodular configuration, which has a significant functional capacity. Hence this will provide a pathway to the development of an effective therapy for the hepatocellular injury. Currently, the treatment for acute liver failure and acute-on-chronic liver failure is liver transplantation[14,17].

LIVER TRANSPLANTATION

Over the years, survival after transplantation has improved with the advances in both patient management and surgical techniques, but the procedure is not always available in a timely fashion[18,19], prompting new surgical approaches, such as split liver transplantation, procurement from living donors and auxiliary liver transplantations[20]. If the transplantation is within the same species then it is called an allograft and if it is across species barriers then it is called as xenogenic transplantation[21-26]. But unfortunately there is a nationwide shortage of organ donors and a large number of patients die before a liver can be procured for transplantation[27]. The problem of organ shortage is compounded by difficulty in predicating the outcome of liver failure[28,29]. Further, the market for liver support is estimated to be substantial: $ 700 million in the United States and $ 1.4 billion worldwide[21]. Therefore, there is an urge to develop an alternative system that could act as a liver support or as a bridge till the donor organ is available or for the native liver regenerates so as to save the lives of the people dying on the waiting list and also to reduce the above mentioned cost for both the government and patients and reap the benefits of a large financial market. This has led to several innovative ideas.

NON-BIOLOGICAL AND BIOLOGICAL APPROACHES

Various non-biological approaches like hemodialysis, hemoperfusion over charcoal, resins, immobilized enzymes, plasmapheresis, and plasma exchanges were explored[14]. But all these have met with limited success, presumably because of the role of the synthetic and metabolic functions of the liver that are inadequately replaced in the above mentioned systems[30]. To overcome these limitations, a biological system should be developed which is likely to succeed only if it performs the function of liver or hepatocytes[24]. The main biological approaches being investigated includes isolated cell transplantation[31-33], tissue engineering of implantable constructs[33-37], transgenic xenotransplantation[38,39] and extra corporeal bioartificial liver devices (BAL).

The artificial liver can be used for several different reasons. First, if the liver is severely damaged and over 80% of the tissue is dead, the artificial liver devise has the capabilities to support the liver until it regenerates completely[14]. If the liver is able to regenerate itself without the need for a transplant, the expensive cost of a liver transplant, which is around $ 2 00 000, can be avoided. Second, the artificial liver device allows for the additional time for the support for a patient waiting on the list of suitable organ. Third, an artificial liver can also be used after a patient has received a transplant to help support that patient until the new graft from the transplantation starts generating new cells.

DEVELOPMENT OF BAL

The issues that have to be considered during the development of bioartificial liver include choice of cellular components, stabilization of hepatocytes phenotype, bioreactor design, regulation, safety and clinical trials.

CELLULAR COMPONENTS OF BAL DEVICES

The cell types that are currently being evaluated for use in BAL are each of these–primary hepatocytes, cell lines, stem cells and xenogenic hepatocytes can be evaluated on the basis of their availability, potential adverse interactions and efficacy in providing liver-specific function. The different types of cellular components present in BAL are summarized in Table 1.

STABILIZATION OF PRIMARY HEPATOCYTES PHENOTYPE

TABLE 1: CELL SOURCES FOR EXTRACORPOREAL BAL DEVICES

Primary cells	Sources
Porcine	Xenogenic, porcine endogenous retrovirus, large scale isolation, environment – dependent function (though some functions are more stable than rodent and human.
Rabbit	Xenogenic, small-scale isolation, environment – dependent, liver – specific functions.
Human	Low availability, heterogeneous donors, environment – dependent function.
Immortalized cells	
C8-B	Rat-SSR69 (SV40T, HSV-TK neoR,LoxP)
HepZ	Human, pCMV. pSV2neo
OUMS-29, NKNT-3	Human fetal, pSVneo or SSR69
HepLiu	Procine, Blue Tag, pRSVneo
Yoon	Human fetal, SV40T
HH25, HHY41	Human, spontaneous
Tumor-derived cells	
Hep G2	Hepatoblastoma
C3A	Hepatoblastoma
HuH6, JHH-2	Hepatoblastoma
Potential stem cell sources	
Embryonic	Derived from blastocyte or germ cells, pluripotent, differentiation to hepatocytes not yet reported *in-vitro*
Progenitor	Oval/progenitor cells are facultative stem cells, hepatoblasts isolated fetal livers are hipotential
Transdifferentiated	Pancreatic ductal cells induces to hepatic lineage, heamopoetic stem cells produce hepatocytes in liver.

Although primary hepatocytes represent the most direct approach to replace liver function in hepatic failure, they must be anchored to a substrate in order to function properly. The various anchoring approaches are as follows:

Encapsulation, a method in which the hepatocytes are enveloped in a polymeric matrix using polysaccharide hydrogels, collagen or other materials so as to maintain a three dimensional structure for the hepatocytes. A number of encapsulation systems have been developed and refined during the past several years in which living cells can be separated from the immune system of the body by a synthetic, selectively permeable membrane[40-46]. The membrane allows the free exchange of nutrients, oxygen and biotherapeutic substances between the blood or plasma and the encapsulated cells, whereas high molecular weight substances, such as immunocytes, antibodies and other transplant rejection effectors mechanism are excluded[47-52]. This system may also modulate the bi-directional diffusion of antigens, cytokines and other immunological moieties based on the chemical characteristics of the membrane and matrix support. Encapsulation cell technology offers a solution to the problem of donor organ supply, not only by potentially allowing the transplantation of cells and tissues without immunosupression but also by permitting use of materials isolated from animals[52-59].

The encapsulation of biological cells by chemically cross-linking the surface of aqueous droplet was modified using milder physical cross-linking[60-63]. This resulted in alginate-polylysine-alginate (APA) microcapsules containing cells. Alginates are heteropolymer carboxylic acids coupled

by 1→4, glycosidic bonds of S-D-mannuramic-(M) and –L-gluconic acid unit. Alkali and magnesium alginate are solvable in water, whereas alginic acids and the salts of polyvalent metal cations are insoluble. Thus, drops of sodium alginate solution entering a calcium chloride solution can form gel spheres. These polymeric materials containing cells are called as microcarriers. Alginate microspheres can be used which can be combined with extra cellular matrix (ECS) material to improve anchorage.

BIOREACTOR DESIGN

BAL devices tailored for use with hepatocytes are becoming a reality coupled with new discoveries in cell sourcing and hepatocytes stabilization. The four main types of bioreactor design that have been proposed and studied are as follows[14].

Hollow fiber:

It is a multi compartmental interwoven fibres with extra capillary seeding and oxygenation so that the cells are protected from shear. Hollow fiber membranes provide a scaffold for cell attachment and immunoisolation, and are well characterized in a clinical setting, but may not provide adequate nutrient transport or the proper environmental cues for long-term hepatocyte stabilization.

Flat plate and monolayer:

It contains a flat membrane reactor with cell in sandwich culture or stacked plate reactor with monolayer culture so as to provide a uniform cell distribution and microenvironment. Flat plate and monolayer bioreactors have been proposed that offer better control of hepatocyte microenvironment, but would be difficult to scale up.

Perfused beds/scaffolds:

It is a microchanneled polyurethane packed bed with spheroids which provides an ease for scale – up and also promotes a three dimensional architecture and minimizes the transport barrier. However, it may be difficult to provide uniform perfusion of the packing matrix and cells can be exposed to damaging shear forces.

Encapsulation:

Hydrogel entrapped cells on rotating disks with perfusion or entrapped aggregates in glass bead packed bed so as to provide an uniform microenvironment and ease of scale-up. A successful extracorporeal bioartificial liver design will include effective bi-directional mass transport, a stable cellular microenvironment, and simple scale-up. Although many devices include a combination of convective and diffusion transport phenomena mass transfer limitations of key nutrients to and from the cellular compartment are primarily due to diffusion resistance. Barriers to diffusive transport include membranes, collagen gels, and nonviable cells. Membranes have been used with a wide range of molecular mass cutoffs, from 20 to 200 kDa, but presently most designs specify a range between 100 and 150 kDa.

BAL CIRCUIT

The encapsulated hepatocytes are then packed in a bioreactor and the BAL circuit can be connected to the patient by using a double lumen catheter, which is placed in the patients, by superficial femoral vein[63,64]. Blood is removed at 90-100 ml/min and separated into blood cells and plasma. The plasma is then perfused through a charcoal column to clear the plasma of low to mid-molecular weight toxic metabolites and allowed the hepatocytes to remain viable for a longer period[65, 66]. However the viability of hepatocytes reported in these systems were upto seven hours[63]. The plasma is then detoxified by passing through the functional hepatocytes and then reconstituted with the patient's blood cells and returned to the patients at the same rate at which it was removed.

PATIENT STATUS

Since the liver is involved in almost all biochemical processes if it shuts down, patients can develop infections, bleeding and brain swelling. When the brain swells, it cuts off blood supply to the brain and causes death. But in a safety trial conducted in The University of Michigan, scientists say that, when the patients were in BAL support there were no adverse mechanical or bleeding effects. The concentrations of ammonia, free fatty acids and amino acid toxins decreased significantly. All patients had improvement in their neurological state, some had decreases in intracranial pressure and there were some signs that the native livers were regaining their ability to produce important molecules of their own[21,22].

REGULATIONS AND SAFETY[14]

Current devices are being regulated as drugs through The Center for Biologics and Evaluation Research of the Food and Drug Administration. New guidelines for these and other hybrid devices are being developed by a consensus based group at the American Society of Testing and Materials in conjunction with other organizations such as the International Standards Organizations. Because of their application to ALF patients for whom other therapies do not exist, some devices have undergone fast-tract review as

TABLE 2: CLINICAL STATUS OF EXTRACORPOREAL SUPPORT DEVICES

Company	No. of patients	Phase	Comments
Vitagen, La Jolla, CA	25	I/II; multicenter	C3A cell line, continuous treatment up to 10 d, ultrafiltrate perfusion, 150–300 ml/min, heparin, 4 replaceable cartridges, cell mass: 4x200
Circe Biomedical, Lexington, MA (HepatAssist)	171	II/III; multicenter	Cryopreserved porcine, treatment 3–6 h for 1–5 d, 400 ml/min, citrate, charcoal (HepatAssist) column, centrifugal plasmapheresis, cell mass: 50 g
Circe Biomedical, Lexington, MA (HepatAssist)	171	II/III; multicenter	Primary porcine, treatment 6–30 h, whole blood perfusion, heparin anticoagulation, cell mass: 100 g
Charite Virchow Clinic–Berlin (MELS)	171	II/III; multicenter	Primary porcine, continuous treatment up to 3 days, filtration plasmapheresis, 100 ml/min, heparin anticoagulation, cell mass: 500 g

orphan drugs. The safety concerns for BAL devices are similar to those for other cellular therapies and include immune reactions for foreign antigens, xenozoonosis and escape of tumorigenic cells.

CURRENT CLINICAL STATUS

Extracorporeal devices are first on the track to clinical application, although their efficacy has yet to be fully determined. Experimental devices using suspended primary hepatocytes were among the first to be used with human patients in the late 1980s, but have met with limited success. Presently, several hollow fiber devices are under evaluation in clinical trials (Table 2)[67-72]. The most extensively tested device, the HepatAssist System from Circe Biomedical (Lexington, MA), completed phase II/III trials with patients. Preliminary results show improvement in 30-day survival to 71% for treated groups, compared with 62% for those receiving standard care (*n* 5). Although an examination of study subpopulations and secondary end points shows moderate benefit of the device, a conclusive measure of efficacy is confounded by factors such as transplantation, disease etiology, and stage of encephalopathy. Critical evaluation of the complete results of the HepatAssist trial should provide valuable insight for future large-scale clinical studies. Careful consideration needs to be given to treatment indications, clinical end points, and device regulation in clinical trial design so that clear evidence of treatment efficacy may be established. Ongoing clinical experiences with extracorporeal support will likely play a key role in the improvement of next-generation devices. Cell transplantation and implantable constructs have thus far seen limited use clinically. Although cell transplantation studies are ongoing in many animal models, only a few investigators have used them in humans to compensate for acute liver failure. To date, there has been no report of the use of a tissue-engineered construct to treat liver disease in humans. As discussed, hepatocyte transplantation and tissue constructs face issues of optimizing transplantation site, nutrient supply, cell viability, and grafting efficiency before clinical safety and efficacy can be evaluated.

ONGOING CLINICAL TRIALS FOR EXTRACORPOREAL BIOARTIFICIAL DEVICES

At least six such systems are undergoing clinical trials. ELAD[67] [Extracorporeal liver assist device; Vitagen], ELAD[68] [Extracorporeal liver assist device; Hepatix], HepatAssist[69] [Circe Biomedical], Liverx2000[70] [Algenix], BLSS[71] [Bioartificial Liver Support System; Excorp Medical, Minneapolis], MLES[72] [Modular Extracorporeal Liver Support; Charite Virchov Clinic-Berlin, Berlin] have been tested on acute or acute-on-chronic liver failure.

CONCLUSIONS

The standard treatment for liver failure has been whole organ transplantation since 1980s. Improvements in surgery have allowed split liver and liver-related donor procedures to partially alleviate the shortage in organ supply. However, cell based therapies provide an important adjunctive treatment (i.e., bridge to liver transplantation) or eventual curative therapy in cases of metabolic defects. Current cell-based approaches will rely on a variety of cell sources, whether primary or stem cells, which will ultimately interact with the microenvironment en route in providing key liver-specific functions. A fundamental understanding of the cues

that promote phenotypic stability and tissue morphogenesis will undoubtedly contribute to the next generation of extracorporeal devices, cell transplantation therapies, and tissue-engineered constructs. Furthermore, strategies to harness and regulate host liver regeneration could even offer the potential to reverse chronic liver fibrosis and cirrhosis, currently thought to be irreversible. In addition, immunological issues will be an important consideration for cell-based therapies; therefore, contributions from transplantation immunology that aim to promote graft tolerance are of great interest. Finally, development of predictive animal models to evaluate liver therapies will offer vital preclinical assessment of new therapies as they emerge.

REFERENCES

1. Gerald, J.T. and Nicholas, P.A., In; Principles of Anatomy and Physiology, Biological Sciences Text books Inc., New York, 1984, 606.
2. Cyril, A.K. and Neil, E., In; Samson Wright's Applied Physiology, Oxford University Press, England, 1971, 420.
3. Donald, E.S., Colin, R.P., Thomas, S. and Nicholas, W.R., In; Text Book of Physiology, Longman Group UK Ltd, Edinburgh, 1988, 280.
4. Sujit, K.C., In; Concise Medical Physiology, New Central Book Agency (Pvt.) Ltd, Kolkata, 2001, 90.
5. Loren, K., In; Perspectives in Human Biology, Wadsworth Publishing Company, Belmont, CA, 1998, 214.
6. Robert, M.B. and Mathew, N.L., In; Principles of Physiology, an Imprint of Mosby – Year Book Inc, USA, 1990, 391.
7. Kiley, J.E., Welsh, H.F., Perder, J.C. and Welch, C.S., **Proc. Soc. Exp. Biol. Med.,** 1956, 91, 489.
8. Schechter, D.C., Nealson, T.F. and Gibbon, J.H., **Surgery,** 1985, 44, 892.
9. Remer, P., Bader, A. and Weissleder, **J. Med. Res. Inst.,** 1998, 3, 687.
10. Nyberg, S.L., Hardin, J.A., Matos, L.E., Rivera, D.J., Misra, S.P. and Gores, G.T. **Surgery,** 2000, 127, 447.
11. Werner, A., Duvar, S., Muthing, J., Buntemeyer, H., Lunsdorf, H., Strauss, M. and Lehmann, J. **Biotechnol. Bioeng.,** 2000, 18, 59.
12. Kawazoe, Y., Eguchi, S., Sugiyama, N., Yuzawa, H., Kawashita, Y., Fujioka, H. and Kanematsu, T., **Cell Transplant.,** 1999, 8, 419.
13. Kong, SB., Chen, S., Demetriou, A.A. and Rozga, J., **Int. J. Artif. Organs,** 1996, 19, 1, 72.
14. Allen, W.J., Hassanein, T. and Bhatia, N.S., **Hepatology,** 2001, 34, 447.
15. Cattral, M.S. and Levy, G.A., **N. Eng. J. Med.,** 1994, 330, 268.
16. Popper, H., In; Leevy, M.C., Eds., The Medical Clinics of North America, Vol. 63, W.B. Saunders Company, Philadelphia, 1979, 479.
17. Bismuth, H., Samuel, D., Casting, D., Williams, R. and Pereira, S.P., **Semen. Liver Dis.,** 1996, 16, 415.
18. Livensky, N.G., **N. Engl. J. Med.,** 2000, 343, 430.
19. Riverea-Penera, T., Moreno, J., Skaff, C., Mc Diarmid, S., Vargas, J. and Ament, M.E., **J. Paediatr. Gastroentrol. Nutr.,** 1997, 24, 128.
20. Goss, J.A., Shackleton, C.R., Maggard, M., Swenson, K., Seu, P., Mc Diarmid, S.V. and Busuttil, R.W., **Arch. Surg.,** 1998, 133, 839.
21. Starl, T.E., **Transplant. Proc.,** 1993, 25, 15.
22. Koebe, H.G., Phaernik, S.A., Sproede, M., Thasier, W.E. and Schildberg, F., **Amer. Soc. Artif. Internal Organs J.,** 1995, 41, 189.
23. Donato, M.J., Bassi, A.M., Gomez-Lechon, M.J., PenCo, S., Herrero, E. and Adamo, D., **In Vitro Cells Dev. Biol.,** 1994, 30A, 574.
24. Nyberg, S.L., Shatford, R.A., Payne, W.D., Hu, W.S. and Cerea, F.B., **Cytotechnology,** 1992, 10, 205.
25. Lazar, A., Mann, H.J., Remmel, R.P., Shatford, R.A., Cerea, F.B. and Hu, W.S., **In Vitro Cells Dev. Biol.,** 1993, 31, 340.
26. Naik, S., Trenkler, D., Santangini, H., Pan, J. and Jaurequi, H., **Cell Transplant.,** 1993, 5, 107.
27. Neuberger, J., Shorrock, C., General considerations. In; Neuberger, J., Lucey, M.R., Eds. Liver Transplantation: Practice and Management, BMJ Publishing Group, London, 1994, 11.
28. Anand, A.C., Nightingale, P. and Neuberger, J.M., **J. Hepatol.,** 1997, 26, 62.
29. Pauwels, A., Mostefa-Kara, N., Florent, C. and Levy, V., **J. Hepatol.,** 1993, 17, 124.
30. Takahashi, T., Malchesky, P.S. and Nose, Y., **Dig. Dis. Sci.,** 1991, 36, 1327.
31. Anand, A.C. and Puri, P., **Indian J. Gasteroentrology.,** 2002, 21, 55.
32. Strom, S.C., Fischer, R.A., Rubinstein, W.S., Barranger, J.A., Towlin, R.B. and Charron, M., **Transplant. Proc.,** 1997, 29, 2103.
33. Rhim, J.A., Sandgren, E.P., Degan, J.L., Palmiter, R.D. and Blinster, R.L., **Science,** 1994, 263, 1149.
34. Mooney, D.J., Kaufmann, P.M., Sano, K., McNamara, K.M., Vacanti, J.P., Langer, R., **Transplant. Proc.,** 1994, 26, 3425.
35. Khanna, J.H., Glenn, J.G., Klen, M.D., Matthew, H.W.T., **Tissue Engineering,** 2000, 6, 670.
36. Dixit, V., Arthur, M., Reinhardt, R. and Gitnick, G., **Artif. Organs.,** 1992, 16, 336
37. Demetriou, A.A., Reisner, A., Sanchez, J., Levenson, S.M., Moscioni, A.D. and Chowdhury, J.R., **Hepatology,** 1988, 8, 1006.
38. Habibullah, C.M., **Gasteroenterol. Today,** 1997, 1, 299.
39. Schmoeckal, M., Bhatti, F.N.K., Zaidi, A., Cozzi, E., Pinnachavez, G., Dunning, J.J. and Wallwork, J., **Transplant. Proc.,** 1997, 29, 3157.
40. Chang, T.M.S., In; Microcapsules and Nanoparticles in Medicine and Pharmacology, CRC Press, USA, 1992, 323.
41. Chang, T.M.S., **J. Bioengineering,** 1, 2532.
42. Jalil, R., Nixon, J.R., **J. Microencaps.,** 1990, 7, 297.
43. Chang, T.M.S., Cofey, J.F., Barre, P., Gonda, A., Dirks, J.H., Levy, M. and Lister, C., **Can. Med. Assoc. J.,** 1993, 108, 429.
44. Chang, T.M.S., **Lancet,** 1972, 13712.
45. Chang, T.M.S., **Artif. Intern. Organs,** 1980, 26, 546.
46. Soonshiong, P., Otterlie, M., Skjak Bracek, G., Smidrod, O.,

Heintz, R., Lanza, R.P. and Espevik, T., **Transplantation,** 1991, 23, 758.
47. Wong, H. and Chang, T.M.S., **Int. J. Artif. Organs,** 1986, 9, 3356.
48. Wong, H. and Chang, T.M.S., **J. Biomat. Artif. Cells. Artif. Organ.,** 1988, 16, 731.
49. Bruni, S. and Chang, T.M.S., **J. Biomat. Artif. Cells. Artif. Organ.,** 1989, 17, 403.
50. Bruni, S. and Chang, T.M.S., **Int. J. Artif. Organs.,** 1991, 14, 239.
51. Dixit, V., Darvasi, R., Arthur, M., Brezina, M., Lewin, K. and Gitnick, G., **Hepatol.,** 1990, 12, 1342.
52. Garofola, F., Chang, T.M.S., **J. Biomat. Artif. Cells. Artif. Organ.,** 1989, 17, 27190.
53. Goosen M.F.A., King G.A., McKnight, C.A. and Marcotte, N., **J. Mem. Sci.,** 1989, 40, 233.
54. Chang, T.M.S. and Poznansky, M.J., **Nature,** 1968, 212, 243.
55. Chang, T.M.S., **Nature,** 1971, 229, 117.
56. Palmour, R.M., Goodyear, P., Reade, T. and Chang, T.M.S., **Lancet,** 1989, 2, 6878.
57. Prakash, S. and Chang, T.M.S., **Nature Medicine,** 1996, 2, 883.
58. Chang, T.M.S. and Prakash, S., **Molecular Medicine Today,** 1998, 4, 221.
59. Chang, T.M.S., **Artif. Organs.,** 1992, 16, 71.
60. Chang, T.M.S., Mac Intosh, F.C. and Mason, S.G., **Can. J. Physical. Pharmacol.,** 1966, 44, 115.
61. Lim, F. and Sun, A.M., **Science,** 1980, 210, 908.
62. Goosen M.F.A., O'Shea, G.M., Gharapetian, H.M., Chou, S. and Sun, A.M., **Biotechnol. Bioeng.,** 1985, 27, 146.
63. Moldeus, P., Hogberg, J. and Sten Orrenius, S., In; Methods in Enzymology, Vol. 77, Academic Press Inc., 1981, 60.
64. Hynie, S., Kamenikova, L. and Farghali, H., In; Bickerstaff, G.F., Eds., Methods in Biotechnology, Vol. 1, Humana Press Inc, Totowa, NJ, 1996, 185.
65. Kawanishi, H., Tsuchiya, T., Hirabayashi, A., Shinhara, R. and Yamanoue, M., **Biomater. Artif. Cells. Artif. Organs,** 1990, 18, 535.
66. Nuzil, D.F., Rozga, J., Moscioni, A.D., Man-Soor, Hakim, R. and Arnaout, W.S., **Surgery,** 1993, 113, 340.
67. Sussanen, N.L., Gisalon, G.T. and Kelly, J.H., **J. Clin. Gastroenterol.,** 1994, 18, 320.
68. Ellis, A.J., Hughes, R.D., Wendon, J.A., Dunne, J., Langley, P.G., Kelly, J.H. and Gislason, G.T., **Hepatology,** 1996, 24, 1446.
69. Rozga, J., Podesta, L., Lepage, E., Morsiani, E., Moscioni, A.D., Hoffman, A. and Sher, L., **Ann. Surg.,** 1994, 219, 538.
70. Nyberg, S.L., Shirabe, K., Peshwa, M.V., Sielaff, T.D., Crotty, P.L., Mann, H.J. and Remmel, R.P., **Cell Transplant.,** 1993, 2, 441.
71. Patzer, J.F., Mazariegos, G.V., Lopez, R., Molmenti, E., Gerber, D., Riddervold, F. and Khanna, A., **Ann. N.Y. Acad. Sci.,** 1999, 875, 340.
72. Gerlach, C., Encke, J., Hole, O., Muller, C., Ryan, C.J. and Neuhaus, P., **Transplantation.,** 1994, 58, 984.