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Review article

Pharmaceutical and therapeutic applications of artificial cells including microencapsulation

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Abstract

Artificial cells for pharmaceutical and therapeutic applications started as microencapsulation on the micron scale. This has now expanded up to the higher range of macrocapsules and down to the nanometer range of nanocapsules and even to the macromolecular range of cross-linked hemoglobin as blood substitutes. This author first reported microencapsulation of biologically active material in 1957 (T.M.S. Chang, Hemoglobin corpuscles. Research Report for Honours Physiology, Medical Library, McGill University, 1957. (Also reprinted as part of 30th anniversary in Artificial Red Blood Cells Research, J. Biomater. Artif. Cells Artif. Organs 16 (1988) 1–9.) and 1964 (T.M.S. Chang, Semipermeable microcapsules, Science 146 (1964) 524–525). While pharmaceutical research has made use of these approaches for drug delivery, this author has been concentrating on the encapsulation of biotechnological products for therapeutic applications. Therefore, there was little interaction between the two approaches. In the last 10 years, pharmaceutical research, as in other areas of research, has become increasingly interested in biotechnology. Because of this interest, this article is a brief overview of developments of artificial cells for biotechnological products with emphasis on hemoglobin, enzymes, cells and genetically engineered microorganisms. © 1998 Elsevier Science B.V.

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1. The first preparation of artificial cells based on microencapsules containing biologically active materials

In 1957, this author reported methods for microencapsulation of biologically active materials [1]. In this initial study, the material for encapsulation was hemoglobin, enzymes and other contents of red blood cells. Three basic approaches were described in this 1957 report.

1. A spray-dry method was first used. Here, the contents were in the form of either powder or a liquid spray. This was sprayed against another spray of polymer solution consisting of collodion (cellulose nitrate in alcohol–ether). A coating can be formed with this process. This was the first reported spray-dry method of encapsulation of a biologically active material.
2. Another approach studied was based on forming a w/o emulsion of the aqueous phase containing the biological active material in an organic phase of ether. This was followed by the addition of collodion. The concentration of the collodion solution was adjusted so that the polymer would precipitate at the w/o interface. This process formed a membrane around each of the microdroplets of the aqueous phase to form microcapsules. The microcapsules were then separated from the organic phase and suspended in an aqueous phase of NaCl solution. The diameter of the microcapsules can be varied between 40 and 200 μm. The procedure is mild since hemoglobin encapsulated retained its

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ability to carry and release oxygen with a normal dissociation curve. This emulsion encapsulation process forms the basis of many of the present microencapsulation approaches.

3. A third approach was based on a drop method. In this approach, a polymer solution (collodion) was layered on top of an organic liquid (liquid paraffin). A drop of the aqueous biological material was released on top of the polymer solution. As it passed through the polymer solution, a polymer coating was deposited around the drop. The polymer coated drop continued to fall through the interface into the organic liquid (paraffin oil). Here the membrane solidified to form a permanent encapsulating membrane. This resulted in the formation of larger 'macrocapsules' up to the millimetre range. The drop method forms the basis of many of the present drop methods for the encapsulation of cells, microorganisms and other materials.

2. Development of methods for artificial cells containing biologically active materials

After medical school and clinical training, this author continued with full time research on the above topic. First, more detailed studies were carried out to analyze in detail the methods of preparation, variations in diameter, effects of emulsifier, use of other membrane materials [2–8]. Double emulsion methods were developed to allow the use of cellulose acetate, polystyrene and other polymer membranes [3]. Interfacial polymerization for forming polyamide membrane was added to the interfacial precipitation method [2–4]. This allows for further variations of membrane material. Extension of this also allow the preparation of cross-linked protein membrane microcapsules [2–4]. Further extension of this resulted in cross-linked hemogolobin [2–4]. Extension also resulted in the preparation of microcapsules with silastic (silicone rubber) membrane [5]. Smaller microcapsules were encapsulated inside larger ones to form multicompartmental systems [3,4]. Cells were encapsulated inside cross-linked protein membrane using silicone oil as the organic phase to prevent adverse effect of organic solvents on the cells [3,4]. Lipid membrane microcapsules were also formed by complexing cholesterol–phospholipid to the surface of microcapsules [6].

The 1964 publication in Science [2] stimulated some interest. Major interest began after the publication of the book on Artificial Cells in 1972 [7]. This resulted in further developments from many laboratories. First reference cited on different new approaches can be found in Table 1.

3. Drug delivery

As discussed earlier, this topic has been of particular interest to pharmaceutical scientists. Biodegradable or biological materials are now being extensively investigated by researchers in drug delivery systems in the form of microcapsules and nanocapsules. Different materials have been used. The use of lipids is

Table 1
First report of methods for artificial cells containing biologically active materials: variations in membranes, configurations and contents

1957 Chang	Microcapsules containing enzyme, multienzymes, proteins—interfacial precipitation, spray dry and drop method
1964 Chang	Microcapsules containing biologically active materials prepared by interfacial polymerization
1964 Chang	Cross-linked protein membrane artificial cells containing single enzyme, multienzymes, protein
1964 Chang	Cross-linked protein–enzyme conjugates and polyhemoglobin
1965 Chang	Artificial cells containing smaller artificial cells for intracellular multicompartmental systems
1965 Chang	Artificial cells containing intact biological cells
1965 Chang	Solid polymer microspheres containing enzymes and biological materials
1966 Mosbach	Solid polymer microspheres containing microorganisms
1966 Chang	Artificial cells containing protein and magnetic material
1966 Chang	Artificial cells containing both enzyme and adsorbent for product of enzyme reaction
1967 Chang	Heparin-complexed membrane artificial cells
1968 Mueller et al.	Bilayer lipid membrane artificial cells containing hemoglobin
1969 Chang	Ultrathin polymer membrane coated adsorbent particles for use in patients
1969 Chang	Bilayer lipid–protein or bilayer lipid–polymer membrane artificial cells containing hemolysate
1970 Sessa and Weissman	Multilamellar lipid liposome containing enzymes
1972 Gregoriadis and Ryman	Multilamellar lipid liposomes as enzymes carrier
1972 May and Li	Liquid hydrocarbon emulsion containing enzyme microdroplets
1973 Ihler	Use of red blood cells as carrier to entrap biologically active materials
1976 Chang	Biodegradable synthetic polymer membrane (e.g. polylactic acid) artificial cells containing hormones (e.g. insulin) and other biologically active materials
1980 Johnston and Chapman	Polymerized liposomes as carrier
1980 Lim and Sun	Alginate–polylysine artificial cells containing islets
1980 Chang	Ultrathin blood compatible polymer coated immunosorbent for removal of antibodies from blood in animal study

Reference [17] contains the detail journal sources of the above references.

Table 2
Pharmaceutical and medical applications of artificial cells

Acute poisoning—routine clinical treatment
Aluminium and iron overload—routine clinical treatment
End-stage kidney failure—routine clinical treatment as supplement to dialysis
Liver failure—routine clinical application for limited types of acute liver failure
Red blood cell substitutes for transfusion—phase III clinical trial
Enzyme defects in inborn errors of metabolism—clinical trial
Monoclonal antibodies production from hybridomas
Diabetic mellitus (bioencapsulated islets)—phase I clinical trial
Bioartificial liver (bioencapsulated hepatocytes)
Gene therapy using bioencapsulated cells or microorganisms
Drug delivery systems
Conversion of cholesterol into carbon dioxide—experimental
Bilirubin removal—experimental
Production of fine biochemical—industrial application
Conversion of wastes (e.g. urea and ammonium) into useful products (e.g. essential amino acids)
Other biotechnological and medical applications—in progress

a common approach. This includes the use of lipid–protein membranes [6], concentric lipid membranes and submicron ultrathin lipid membranes [9]. Another approach is biodegradable synthetic polymer. The first one used for microencapsulation was polylactide [10]. Many types of polylactides and polyglycolic acids are being used at present [11]. Other synthetic biodegradable polymers have also been investigated. Polyanhydride is one example [12]. Biodegradable microcapsules and nanocapsules encompass a very active field of research and since this area is familiar to researchers in the pharmaceutical sciences, it will not be reviewed here.

4. Artificial cells

In biotechnological approaches, the use of artificial cells is based on a slightly different principle than for drug delivery. Artificial cells are prepared to permanently retain proteins, enzymes, cells, microorganisms or adsorbents. The biologically active material is permanently retained inside the particle and not released. While inside, the enclosed materials act on smaller molecules that can rapidly penetrate into the artificial cells. In addition, products from the enclosed materials (e.g. insulin from encapsulated islets) can be released readily. In this way, artificial cells containing (1) adsorbents can remove toxins. (2) Hemoglobin can transport oxygen. (3) Enzymes can convert or remove metabolites and substrates. (4) Cells (e.g. islets and hepatocytes) and genetically engineered microorganisms can carry out useful functions. The present ongoing research and clinical applications are shown in Table 2. This is now a very large area with many groups carrying out excellent studies. This brief overview cannot be a complete overview of all groups and all areas. Instead, it will summarize several important areas being carried out by groups around the world, and illustrated with details from our research.

5. Artificial cells in acute poisoning, aluminium poisoning, renal and liver failure

Our laboratory and clinical research on microencapsulated activated charcoal for hemoperfusion [13–15] has led to its routine clinical applications [16], Microencapsulation of activated charcoal prevents the release of charcoal powder. It also prevents the activated charcoal from having an adverse effect on blood cells. At the same time, the ultrathin coating membrane is prepared to have high permeability to smaller molecules [13,14]. Charcoal (70 g) is retained in a column that is connected to the circulation of the patients for 2 h in each treatment. As blood from the patient passes through the column, the encapsulated activated charcoal removes toxins, drugs and waste metabolites. This has led to its routine use for patients for acute poisoning, aluminium removal and as supplement to renal failure and liver failure treatment [14–17]. This procedure is now being used routinely especially for the treatment of acute poisoning.

6. Modified hemoglobin as blood substitutes

In 1985, concerns of HIV donor blood for transfusion stimulated research into blood substitutes. Interest therefore returned to this author's original approach of cross-linked hemoglobin and artificial cells encapsulated hemoglobin [1–8]. In the last 10 years, work carried out by many groups including us, have resulted in significant progress in this area [18–22] Industries are successfully developing cross-linked ultrapure hemoglobin and these products are now in clinical trials for patients. One group has infused up to six units (3 l) of cross-linked polyhemoglobin (10 g/dl) into patients to replace an equivalent amount of blood loss. These researchers are proceeding to Phase III clinical trials using up to ten units (5 l). A second generation blood substitute has resulted from the cross-linking of hemoglobin with trace amounts of superoxide dismutase and catalase [22]. This is used to prevent reperfusion injury due to the formation of oxygen radicals. Microencapsulated hemoglobin with lipid membranes or biodegradable nanocapsules are being developed as the third generation blood substitutes [22].

7. Artificial cells containing an enzyme system

Earlier study demonstrated the effectiveness of implantation of artificial cells containing enzymes for inborn errors of metabolism and for lymphosarcoma [23–25]. This approach also prevented problems related to hypersensitivity, immunology and instability [23–25]. However, repeated injections are needed and the problems of retention of foreign materials must be resolved. We therefore investigated the oral administration approach. A patient with Lesch–Nyhan disease was treated by oral administration of artificial cells containing xanthine oxidase to lower systemic hypoxanthine [26,27]. This is an inborn error of metabolism due to hypoxanthine phosphoribosyntransferase deficiency. Hypoxanthine being lipid soluble diffuses rapidly from blood into the intestinal tract where it is removed by the enzyme artificial cells. Within 1 week, there was a fall in plasma hypoxanthine level. There was also a reduction of CSF hypoxanthine after 2 weeks and there were no adverse effects. Since this is an extremely rare disease, we are studying a more common inborn-error of metabolism, Phenylketonuria (PKU). We started with a phenylketonuria PKU rat model using artificial cells containing phenylalanine ammonia–lyase [28]. This successfully lowered the systemic phenylalanine levels. The treated PKU rats grew and gained weight. The untreated group continued to have high systemic phenylalanine levels and lost weight. The clinical implication of these results only became obvious after our finding of a large recycling of amino acids between the intestinal content and the body-enterorecirculation [29]. This finding allows the use of microcapsules containing any one specific enzyme to remove a specific unwanted amino acid from the body by oral administration. This is now being developed for clinical trials for PKU patients, and later, for the removal of specific unwanted amino acids in other conditions.

8. Artificial cells containing cells

As discussed earlier, this author used a drop method to encapsulated cells inside microcapsules [3,4,7] In his 1965 publication, it was proposed that this method could be used for preventing immunological rejection of implanted endocrine cells and hepatocytes, for example as follows [3]:

> '....microencapsulation of intact cells or tissue fragments... the enclosed material might be protected from destruction and from participation in immunological processes, while the enclosing membrane would be permeable to small molecules of specific cellular product which could then enter the general extracellular compartment of the recipient. For instance, encapsulated endocrine cells might survive and maintain an effective supply of hormone... The situation would then be comparable to that of a graft placed in an immunologically favourable site.' 'There would be the further advantage that implantation could be accomplished by a simple injection procedure rather than by a surgical operation.'

However, it was not until the 1980s that with the persistence of the author that this procedure was finally developed by the Canaught laboratory [30]. Thus Sun's group from this company, carried out extensive research to modify and extend the cell encapsulation method for islets [30]. They were able to use this method to treat diabetic rats and to maintain a normal glucose level after implantation of these artificial cells. Other groups soon followed. Thus, one group from the US has progressed to the point where they have carried out FDA approved clinical trails for patients [31]. Other groups including ourselves have also studied the encapsulation of other endocrine cells, hepatocytes and other cells. We also showed that artificial cells containing hepatocytes are useful as liver support for liver failure [32] and in the Gunn rat [33,34]. These results have stimulated a number of other groups to carry out research in cell encapsulation [35–39]. The use of artificial cells to encapsulate erythropoietin secreting cells has also been investigated [40]

The microencapsulation of smaller cells such as hepatocytes or microorganisms was not always reproducible. This was due to the entrapment of small cells in the membrane that weakens the membrane integrity. We solved this problem recently by using a novel two step method [41]. This method may be especially useful in view of the potential applications of genetically engineered microorganisms to be discussed below.

9. Artificial cells containing genetically engineered microorganisms

Molecular biology has resulted in genetically engineered microorganisms that can be induced to result in highly specialized and effective enzyme systems. However, genetically engineered microorganisms cannot be injected into the body for therapeutic uses. We therefore studied the microencapsulation of genetically engineered nonpathogenic microorganisms inside permanent polymeric artificial cells for oral administration [42–45]. When given orally the microorganisms remained inside the microcapsules at all times as they pass down the intestine and then excreted with the stool. This way, no microorganism is released into the intestine. During the passage through the intestine, the genetically engineered microorganisms in the microcapsules act on the specific substrate that diffuses into the microcapsules.

We carried out our preliminary studies in a surgical uremic kidney failure rat model [43,44]. When given orally, *E. coli* DH5 bacteria remained inside the microcapsules at all time as the microcapsules passed down the intestine and were then excreted with the stool. This way, no bacteria were released into the intestine. During the passage through the intestine, the genetically engineered *E. coli* DH5 bacteria in the microcapsules act on urea and ammonia diffusing into the microcapsules. This removes the high urea level in kidney failure rats resulting in a return to normal urea levels.

A specific example is as follows. We gave daily oral artificial cells containing 5 mg of *E. coli* with K. aerogenes gene to each kidney failure rat. This resulted in the lowering of the uremic urea level (60.43 ± 10.00) to 21.15 ± 3.36 mg/dl in 3 days. It then fell to 12.86 ± 1.21 mg/dl by the 13th day and remained at this low level during the period of daily oral administration. This is equivalent to the daily oral artificial cells containing 1.5 g of the microorganisms in a 70 kg man. When oral administration was stopped the urea level increased back to pretreatment levels. Blood ammonia was also lowered to normal level. Uremic rats receiving control microcapsules did not have any decrease in blood urea or ammonia levels. None of the treated uremic rats died during the treatment period, whereas 50% of the untreated uremic rats died. The treated uremic rats gained weight at the same rate as the normal control rats. The untreated uremic rats lost weight and died.

Removal of urea by oral delivery of the product suggests that the final obstacle to a possible oral treatment for kidney failure has been overcome, by using a combination of different sorbents and fluid removal systems. With the final obstacle of urea removal solved, there is now the real potential of an oral pharmaceutical approach for kidney failure to replace the expensive and cumbersome treatment using dialysis.

10. General

Thus, artificial cells could be an addition to the numerous pharmaceutical tools available for medical applications. This overview only touches on a few areas with some brief examples being presented. More extensive details are available elsewhere [21,36,38,46–51]. A complete reference listing of more than 400 publications from our group and overview of different areas can be found in our web site: www.physio.mcgill.ca/artcell

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References

[1] T.M.S. Chang, Hemoglobin corpuscles. Research Report for Honours Physiology, Medical Library, McGill University, 1957. (Also reprinted as part of 30th anniversary in Artificial Red Blood Cells Research, J. Biomater. Artif. Cells Artif. Organs 16 (1988) 1–9.)
[2] T.M.S. Chang, Semipermeable microcapsules, Science 146 (1964) 524–525.
[3] T.M.S. Chang, Semipermeable aqueous microcapsules. Ph.D. Thesis, McGill University, 1965.
[4] T.M.S. Chang, F.C. MacIntosh, S.G. Mason, Semipermeable aqueous microcapsules: I. Preparation and properties, Can. J. Physiol. Pharmacol. 44 (1966) 115–128.
[5] T.M.S. Chang, Semipermeable aqueous microcapsules 'artificial cells': with emphasis on experiments in an extracorporeal shunt system, Trans. Am. Soc. Artif. Intern. Organs 12 (1966) 13–19.
[6] T.M.S. Chang, Lipid-ocated spherical ultrathin membranes of polymer or cross-linked protein as possible cell membrane models, Fed. Proc. 28 (1969) 461.
[7] T.M.S. Chang, Artificial Cells, Thomas, Springfield, IL, 1972.
[8] T.M.S. Chang, F.C. MacIntosh, S.G. Mason, Encapsulated hydrophillic compositions and methods of making them, Canadian Patent, No. 873 815, 1971.
[9] F. Gregoriadis, Liposomes as Drug Carriers: Recent Trends and Progress, Wiley, New York, 1989.
[10] T.M.S. Chang, Biodegradable semipermeable microcapsules containing enzymes, hormones, vaccines, and other biological, J. Bioeng. 1 (1976) 25–32.
[11] R. Jalil, J.R. Nixon, Biodegradable poly(lactic acid) and poly(-lactide-co-glycolide) microspheres, J. Microencapsulation 7 (1990) 297.
[12] E. Mathiowitz, R. Langer, Polyanhydride microspheres as drug delivery systems, in: M. Donbrow (Ed.), Microcapsules and Nanoparticles in Medicine and Pharmacology, CRC Press, Boca Raton, FL, 1992, pp. 99–123.
[13] T.M.S. Chang, Removal of endogenous and exogenous toxins by a microencapsulated absorbent, Can. J. Physiol. Pharmacol. 47 (12) (1969) 1043–1045.
[14] T.M.S. Chang, Microencapsulated adsorbent hemoperfusion for uremia, intoxication and hepatic failure, Kidney Int. 7 (1975) S387–S392.
[15] T.M.S. Chang, Hemoperfusion alone and in series with ultrafiltration or dialysis for uremia, poisoning and liver failure, Kidney Int. 10 (1976) S305–S311.
[16] J.F. Winchester, Hemoperfusion, in: J. Maher (ed) Replacement of Renal Function by Dialysis, 3rd ed., Kluwer, Boston, MA 1988, pp. 439–460.
[17] T.M.S. Chang, Biotechnology of artificial cells including application to artificial organs, in: M. Moo-Young, (Ed.), Comprehensive Biotechnology: The Principles, applications and Regulations of Biotechnology in Industry Agriculture and Medicine, Pergamon, New York, 1985, pp. 53–72.
[18] T.M.S. Chang, R. Geyer (Eds.), Blood Substitutes, Marcel Dekker, New York, 1989, p. 714.
[19] T.M.S. Chang, J. Reiss, R. Winslow (Eds.), Blood Substitutes (special issue), Artif. Cells Blood Substit. Immbolization Biotechnol., 22 (1994) 123–360.
[20] T.M.S. Chang, G. Greenberg, E. Tsuchida, (Eds.), Blood Substitutes (special issue), Artif. Cells Blood Substit. Immbolization Biotechnol., 25 (1997) 1–227.

[21] T.M.S. Chang, Blood Substitutes: Methods, Principles, Products and Clinical Trials, vol. 1., Karger/Landes Systems, Georgetown, TX, 1997.
[22] T.M.S. Chang, Recent and future developments in modified hemoglobin and microencapsulated hemoglobin as red blood cell substitutes, Artif. Cells, Blood Substit. Immobilization Biotechnol. 25 (1997) 1–24.
[23] T.M.S. Chang, M.J. Poznansky, Semipermeable microcapsules containing catalase for enzyme replacement in acatalasemic mice, Nature 218 (5138) (1968) 242–245.
[24] M.J. Poznansky, T.M.S. Chang, Comparison of the enzyme kinetics and immunological properties of catalase immobilized by microencapsulation and catalase in free solution for enzyme replacement, Biochim. Biophys. Acta 334 (1974) 103–115.
[25] T.M.S. Chang, The in vivo effects of semipermeable microcapsules containing L-asparaginase on 6C3HED lymphosarcoma, Nature 229 (528) (1971) 117–118.
[26] T.M.S. Chang, Preparation and characterization of xanthine oxidase immobilized by microencapsulation in artificial cells for the removal of hypoxanthine, Biomater. Artif. Cells Artif. Organs 17 (1989) 611–616.
[27] R.M. Palmour, P. Goodyer, T. Reade, T.M.S. Chang, Microencapsulated xanthine oxidase as experimental therapy in Lesch–Nyhan disease, Lancet 2 (8664) (1989) 687–688.
[28] L. Bourget, T.M.S. Chang, Phenylalanine ammonia-lyase immobilized in microcapsules for the depletion of phenylalanine in plasma in phenylketonuria rat model, Biochim. Biophys. Acta 883 (1986) 432–438.
[29] T.M.S. Chang, C. Lister, Plasma/Intestinal concentration patterns suggestive of entero-portal recirculation of amino acids: Effects of oral administration of asparaginase, glutaminase and tyrosinase immobilized by microencapsulation in artificial cells, Biomater. Artif. Cells Artif. Organs 16 (1988) 915–926.
[30] F. Lim, A.M. Sun, Microencapsulated islets as bioartificial endocrine pancreas, Science 210 (1980) 908–909.
[31] P. Soon-Shiong, R.E. Heintz, N. Merideth, Q.X. Yao, Z. Yao, T. Zheng, M. Murphy, M.K. Moloney, M. Schmehll, M. Harris, R. Mendez, P.A. Sandford, Insulin independence in a type I diabetic patient after encapsulated islet transplantation, Lancet 343 (1994) 950–951.
[32] H. Wong, T.M.S. Chang, Bioartificial liver: implanted artificial cells microencapsulated living hepatocytes increases survival of liver failure rats, Int. J. Artif. Organs 9 (1986) 335–336.
[33] S. Bruni, T.M.S. Chang, Hepatocytes immobilized by microencapsulation in artificial cells: Effects on hyperbilirubinemia in Gunn rats, J. Biomater. Artif. Cells Artif. Org. 17 (1989) 403–412.
[34] S. Bruni, T.M.S. Chang, Kinetic analysis of UDP-glycoronosyltransferase in bilirubin conjugation by encapsulated hepatocytes for transplantation into rats, Artif. Org. 19 (1995) 449–457.
[35] V. Dixit, M. Arthur, G. Gitnick, Repeated transplantation of microencapsulated hepatocytes for sustained correction of hyperbilirubinemia in Gunn rats, Cell Transplant 1 (1992) 275–279.
[36] R. Lanza, R. Langer, W.L. Chick, Principles of Tissue Engineering. Academic Press/Landes, Austin, TX, 1997.
[37] W. Stevenson, M.V. Sefton, Development of polyacrylate microcapsules, in: M.F.A. Goosen (Ed.), Fundamentals of Animal Cell Encapsulation and Immobilization, CRC Press, Boca Raton, FL, 1993, pp. 143–182
[38] M.F.A. Goosen (Ed.), Fundamentals of Animal Cell Encapsulation and Immobilization, CRC Press Boca Raton, FL, 1993, pp. 326–362.
[39] D. Poncelet, B. Poncelet, C. Beaulieu, R.J. Neufeld, Scale-up of gel bead and microcapsule production in cell immobilization, in: M.F.A. Goosen (Ed.), Fundamentals of Animal Cell Encapsulation and Immobilization, CRC Press, Boca Raton, FL, 1993, pp. 113–182.
[40] J. Koo, T.M.S. Chang, Secretion of erythropoietin from microencapsulated rat kidney cells: preliminary results, Int. J. Artif. Organs. 16 (1993) 557–560.
[41] H. Wong, T.M.S. Chang, A novel two step procedure for immobilizing living cells in microcapsules for improving xenograft survival, Biomater. Artif. Cells Immobilization Biotechnol. 19 (1991) 687–698.
[42] T.M.S. Chang, S. Prakash, Artificial cells for bioencapsulation of cells and genetically engineered *E. coli*: for cell therapy, gene therapy and removal of urea and ammonia. chap. 75 in: Expression and Detection of Recombinant Genes, in: Methods in Molecular Biology Series, Humana Press, Clifton, NJ, 1997, pp. 343–358.
[43] S. Prakash, T.M.S. Chang, Preparation and in vitro analysis of microencapsulated genetically engineered *E. coli* DH5 cells for urea and ammonia removal, Biotechnol. Bioeng. 46 (1995) 621–626.
[44] S. Prakahs, T.M.S. Chang, Microencapsulated genetically engineered live *E coli* DH5 cells administered orally to maintain normal plasma urea level in uremic rats, Nat. Med. 2 (1996) 883–887.
[45] T.M.S. Chang, Live *E. coli* cells to treat uremia: replies to letters to the editor, Nat. Med. 3 (1997) 2–4.
[46] T.M.S. Chang, Artificial cells, in: R. Dulbecco (Ed.), Encyclopedia of Human Biology vol. 1, Academic Press, San Diego, CA, 1991 pp. 377–383.
[47] T.M.S. Chang, Recent advances in artificial cells with emphasis on biotechnological and medical approaches based on microencapsulation. in: M. Donbrow (Ed.), Microcapsules and Nanoparticles in Medicine and Pharmacology, CRC Press, Boca Raton, FL, 1992, pp. 323–339.
[48] M. Donbrow (Ed.), Microcapsules and Nanoparticles in Medicine and Pharmacology, CRC Press, Boca Raton FL, 1992, pp. 323–339.
[49] T.M.S. Chang, Artificial cells with emphasis on bioencapsuolation in biotechnology, Biotechnol. Annu. Rev. 1 (1995) 267–296.
[50] T.M.S. Chang (Ed.), Artificial Cells, Blood Substitutes and Immobilization Biotechnology, an international journal, Marcel Dekker, New York.
[51] International Society for Artificial Cells, Blood Substitutes and Immobilization Biotechnology, Web site: www.physio.mcgill.ca/artcell.