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Artificial Cells in Medicine and Biotechnology

T. M. S. CHANG

Artificial Cells and Organs Research Centre, Faculty of Medicine, McGill University, 3655 Drummond Street, Montreal, Canada

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ABSTRACT

Since the feasibility of artificial cells was first demonstrated in 1957 [Chang (*1, 2*)], an increasing number of approaches to their preparation and use have become available. Thus artificial cell membranes can now be formed using a variety of synthetic or biological materials to produce desired variations in their permeability, surface properties, and blood compatibility. Almost any material can be included within artificial cells. These include enzyme systems, cell extracts, biological cells, magnetic materials, isotopes, antigens, antibodies, vaccines, hormones, adsorbents, and others. Since cells are the fundamental units of living organisms, it is not surprising that artificial cells can have a number of possible applications. This is especially so since artificial cells can be "tailor-made" to have very specialized functions. A number of potential applications suggested earlier have now reached a developmental stage appropriate for clinical trial or application. These clinical applications include the use of such cells as a red blood cell substitute, in hemoperfusion, in an artifical kidney or artificial liver, as detoxifiers, in an artificial pancreas, and so on. Artificial red blood cells based on lipid-coated fluorocarbon or crosslinked hemoglobin are being investigated in a number of centers. The principle of the artificial cells is also being used in biotechnology to immobilize enzymes and cells. Developments in biotechnology have also resulted in the use of the principle underlying the artificial cell to help produce interferons and monoclonal antibodies; to create immunosorbents; to develop an artifi-

cial pancreas; and to bring enzyme technology usefully into biotechnology and biomedical applications. Artificial cells are also being used as drug delivery systems based on slow release, on magnetic target delivery, on biodegradability, on liposomes, or other approaches. The present status and recent advances will be emphasized in this paper.

Index Entries: Artificial cells; microencapsulation; blood substitutes; immobilized enzyme; immobilized cells; biotechnology; artificial organs; poisoning; immunosorbent; hemoperfusion; charcoal; enzyme; drug carrier; renal failure; liver failure.

INTRODUCTION

In 1956, when I proposed the idea of artificial cells there was so much incomprehension that I had to do the initial research privately at my McGill University residence. Fortunately, the concept very shortly received encouragement and support from Prof. F. C. MacIntosh, Prof. A. V. Burgen, and later Prof. S. G. Mason and others at McGill, allowing me to openly continue and complete the initial part of this research (*1*) during and after medical school (*2–4*). At that time one could not have dreamed of the eventual extent of the present international research activities that incorporate one or more of the principles of artificial cells. Examples include "hemoperfusion," "liposomes," "microencapsulated enzymes," "microencapsulated cells," "magnetic microcapsules," "blood substitutes," "lipid-coated fluorocarbon emulsion," "polyhemoglobin," and others. Since many of the investigators doing this research are presenting their own work in these pages, the present review will concentrate more on studies that have been carried out in our own McGill laboratories.

MEMBRANES OF ARTIFICIAL CELLS (FIG. 1)

Artificial cell membranes can be formed using emulsification followed by membrane formation around each microdroplet by interfacial precipitation (*1–6*) or by interfacial polymerization (*2–9*). Another method is to form a secondary emulsion using silastics, cellulose acetate, and other polymers (*3, 10*) or using liquid membranes (*11*). Still another approach is to apply an ultrathin membrane to form a coating around each protein–enzyme granule (*1*) or adsorbent (*5, 12*). Biological and biodegradable membranes have also been used to form artificial cell membranes. For example, spherical ultrathin crosslinked protein membranes were the first type of biological and biodegradable membrane used (*2–6*). Heparin-complexed polymer membrane can also be used (*13*). Spherical ultrathin lipid membrane artificial cells (*14*) have been prepared using a modification of the method for preparing artificial cells (*2*). However, the ultrathin lipid membranes were not sufficiently stable.

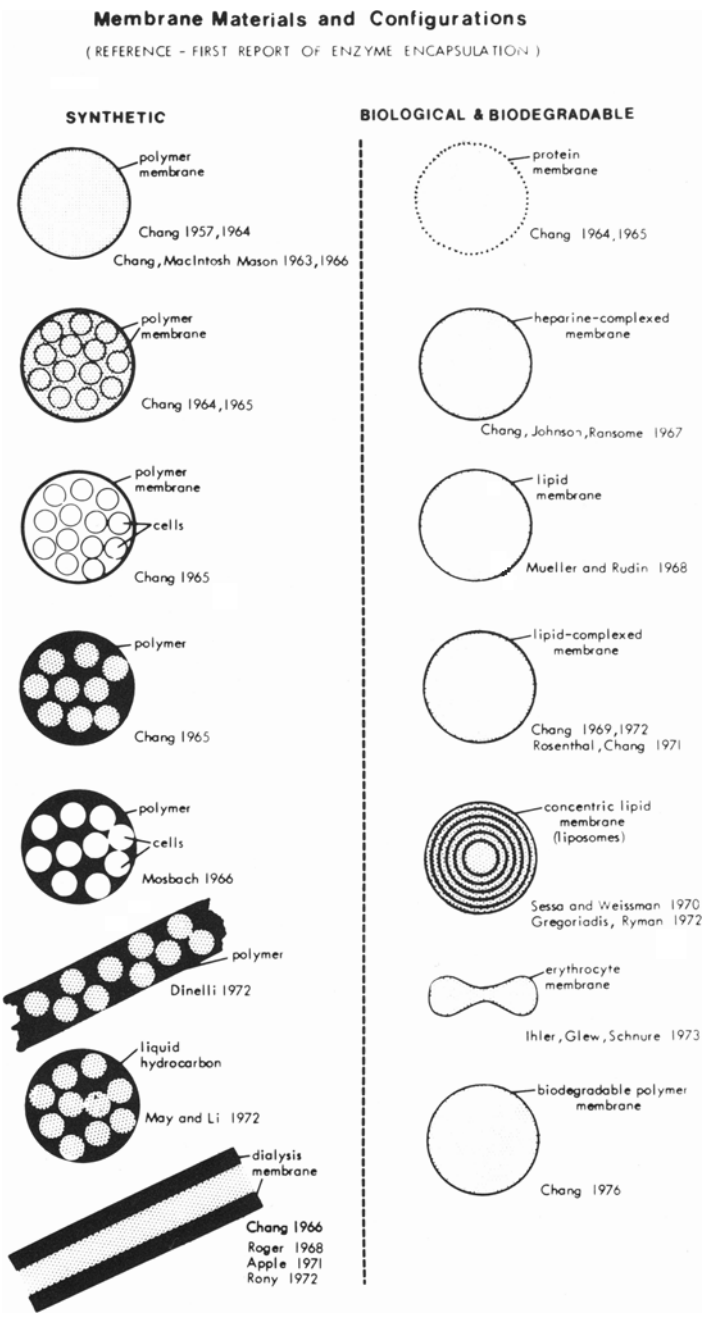

Fig. 1. Membrane materials and configurations. References given are first published reports (*6*).

This problem has been solved by the use of a lipid–protein complexed membrane (*5, 15–17*) or by the use of concentric lipid membranes (liposomes) (*18, 19*), or by the more recent use of very small spherical ultrathin lipid membrane vesicles. The use of erythrocyte-encapsulated enzymes is another approach employing biological membranes (*20*). Synthetic biodegradable membrane, e.g., polyactic acid, has also been used (*21*).

CONTENTS OF ARTIFICIAL CELLS (FIG. 2)

Most materials can be microencapsulated within artificial cells. This includes single enzyme and multienzyme systems, cell extracts, and combined enzyme–adsorbent systems (*1–11, 17–25*). Biological cells have been enclosed to prevent them from being adversely affected by external factors and immunological rejection (*3–6, 26, 27*). Magnetic material has been enclosed to allow the artificial cells to be localized by an external magnetic field (*10, 30*). Other materials include radioisotope-labeled material (*3, 5*); insolubilized enzymes (*5, 28*); crosslinked proteins and enzymes (*2–6, 29*); cofactor recycling multienzyme systems (*31–36*); antigens, antibodies, vaccines, and hormones (*21, 35–38*). Whole artificial cells can be prepared as crosslinked protein systems of different sizes (*2–6, 29, 35, 36*). Adsorbents have also been enclosed within artificial cells for use in detoxification (*5, 10, 12*).

Some typical examples of artificial cells will be discussed in the following sections.

IMMOBILIZATION OF ENZYME AND MULTIENZYME SYSTEMS WITHIN ARTIFICIAL CELLS

Most enzymes function in an intracellular environment acting sequentially on substrates, including those that cross the cell membranes by passive movement or by special transport mechanisms. All enzymes tested so far can be successfully enclosed within artificial cells although some enzymes require modifications of the published methods (*2–6, 35, 36*). A 10 g/dL quantity of hemoglobin encapsulated in the standard artificial cells results in an intracellular environment somewhat comparable to that of red blood cells (*2–6, 29, 35, 36*). In this way, the enzymes enclosed in the artificial cells are stabilized by the high concentration of protein (*29*). Further stabilization can be obtained by crosslinking with glutaraldehyde (*29*).

Most metabolic functions are carried out in cells by complex multienzyme systems with cofactor requirements. Artificial cells have been prepared containing multienzyme systems with cofactor recycling incorporated. Artificial cells containing hexokinase and pyruvate kinase continuously recycle ATP for the conversion of glucose into G-6-P and

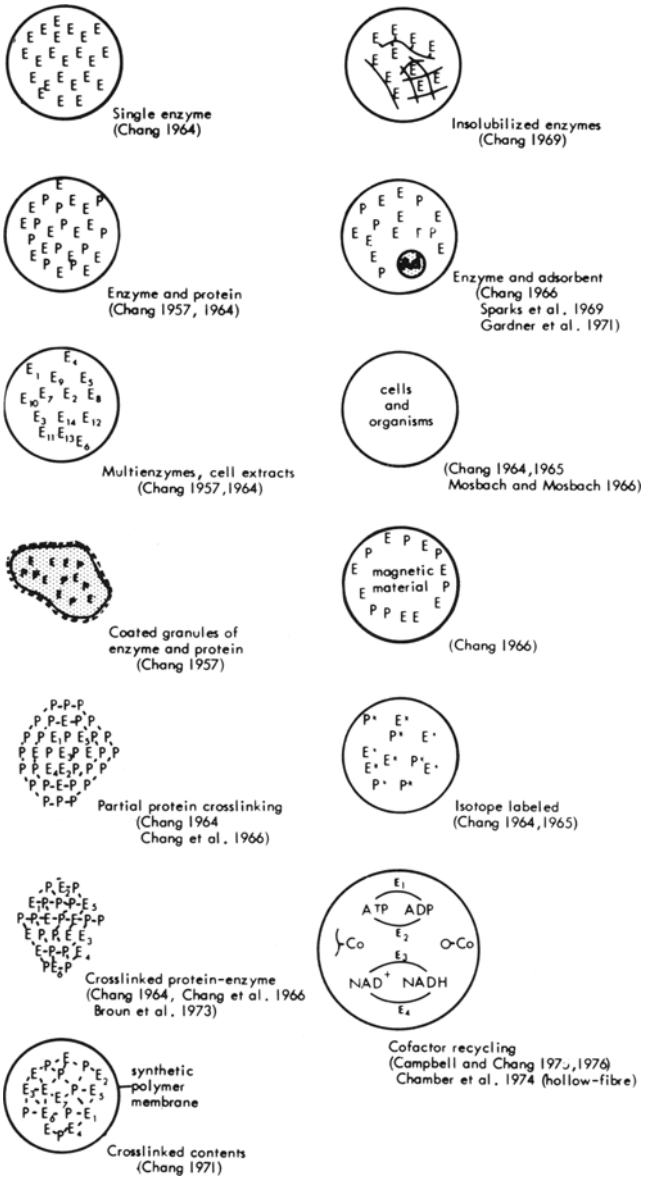

Fig. 2. Variations in contents of artificial cells. References given are first published reports on the subject (*6*).

phosphoenol pyruvate into pyruvate (*31*). Artificial cells containing alcohol dehydrogenase and malic dehydrogenase recycle NADH from of NAD^+ (*32*). A multienzyme system consisting of urease, glutamate dehydrogenase, and glucose-6-phosphate dehydrogenase can be microencapsulated within each artificial cell (*33*). This way, urea can be con-

verted into ammonia, which is then sequentially converted into an amino acid, glutamate. The required cofactor NADPH is recycled by the enzyme glucose-6-phosphate dehydrogenase. Artificial cells containing urease, glutamine dehydrogenase, and glucose dehydrogenase can also be used (*39*). The advantage of this is that glucose instead of glucose-6-phosphate can be used to recycle the required cofactor NADH (*39*). Glutamate formed from urea or ammonia can be further converted into other amino acids by the addition of transaminase to the multienzyme system in the same artificial cell (*40*). Artificial cells containing multienzyme systems have also been studied for galactose conversion (*41*). In these systems a very low external concentration of cofactor is required for continuous recycling of the multienzyme reaction. However, the cofactors should be retained within the artificial cells for in vivo applications or for perfusion systems. One approach is to link cofactors to dextran to form soluble macromolecules. Thus, semipermeable nylon polyethyleneimine microcapsules have been prepared to contain alcohol dehydrogenase, malic dehydrogenase, and soluble dextran–NAD^+ (*34*). This way, dextran–NAD^+, while retained within the artificial cells, can be recycled by the sequential reactions of the microencapsulated enzymes. Another approach for retaining cofactor inside artificial cells for continuous recycling is the use of complexed lipid–polymer membrane. Lipid–polymer complex membrane artificial cells (*5, 15*) have very low permeability to nonlipid soluble molecules, but high permeability to lipid-soluble molecules (*5, 15, 16*). Studies have been carried out using ultrathin lipid–polymer membrane artificial cells containing multienzymes, cofactors, and substrates for multistep enzyme reactions (*17, 42, 43*). The ultrathin lipid–nylon membrane microcapsules can retain enzymes, NAD^+, NADH, and α-ketoglutarate. External ammonia and alcohol can cross the lipid membrane to take part in the multistep reactions. Results show that the cofactors and substrates required for multienzyme reactions can be retained within the ultrathin lipid–nylon microcapsules to act on permeant external substrates (*17, 42, 43*) (Fig. 3).

Artificial cells containing enzymes and proteins have been used in a number of experimental and therapeutic conditions. Some of these are briefly summarized. Microencapsulated urease has been used as a model immobilized enzyme system for experimental therapy (*2–6*). The basic results obtained pave the way for other types of enzyme replacement therapy. The first demonstration for replacement in hereditary enzyme-deficiency conditions is the use of microencapsulated catalase to replace a hereditary catalase deficiency in acatalasemic mice (*44, 45*). Repeated injections did not result in the production of immunological reactions to the heterogenous enzyme in the artificial cells (*45*). The studies on the use of liposome microencapsulated enzymes for experimental replacement in hereditary enzyme-deficiency conditions related to storage diseases have been reviewed elsewhere (*46*). Red blood cell-entrapped enzymes (*20*) have also been tested for possible use in storage diseases.

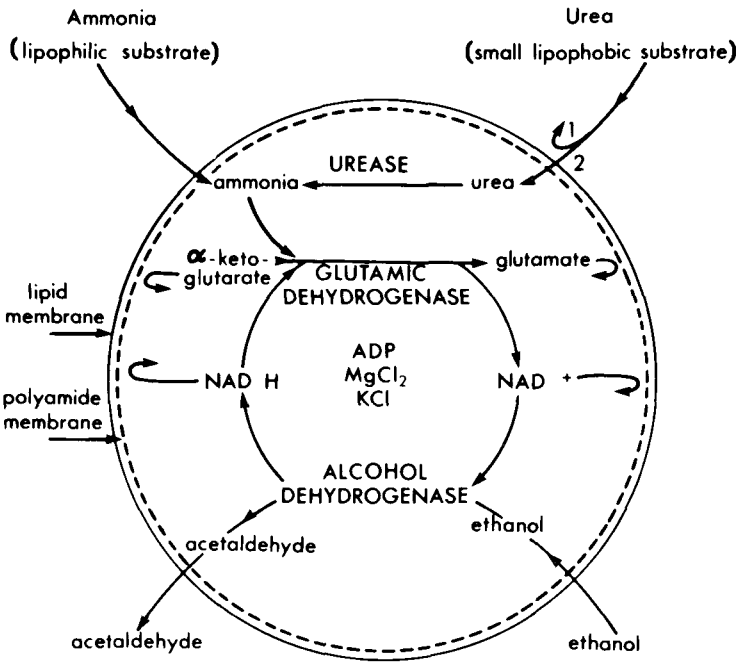

Fig. 3. Example of lipid–polymer membrane artificial cells containing a multienzyme system and the required cofactors (*43*).

Artificial cells containing asparaginase have been used for experimental tumor suppression (*5, 47, 48*). Microencapsulated urease in an extracorporeal hemoperfusion system lowered the systemic blood urea of dogs by 50% within 45 min (*10*). The ammonium ion formed was removed by microencapsulated ammonium adsorbent (*10*). This principle of urea removal using urease and ammonium adsorbent (*10, 28*) was later successfully adapted into the Redy system for urea removal in dialyzate. A combined urease and ammonia adsorbent system has also been investigated for the possible removal of urea by oral administration (*5, 24, 25, 28, 49, 50*). Artificial cells containing tyrosinase have been prepared and tested for hemoperfusion in galactosamine-induced fulminant hepatic failure (FHF) rats (*51*). It was found that hemoperfusion through tyrosinase artificial cells resulted in a significant lowering of tyrosine in systemic circulation (*51, 52*).

ARTIFICIAL CELLS CONTAINING BIOLOGICAL CELLS

Artificial cells containing biological cells have been prepared here (*3–6*). It was proposed that in this form, cells (e.g., endocrine cells) can be implanted since they will be prevented from rejection by the immunological system of the body (*3–6*). The feasibility for this potential has already been indirectly demonstrated in the implantation of artificial

containing enzymes for successful in vivo action without causing immunological reactions (*5, 6, 44, 45, 47, 48*). Further recent developments elsewhere, especially in the improvement of our original microencapsulated cell technology (*3–6*) have resulted in the possibility of microencapsulating living biological cells that can be maintained in culture (*27, 38*). This has made it possible for rat islet cells to be microencapsulated and then implanted intraperitoneally into diabetic rats (*27*). Artificial cells containing fibroblasts or plasma cells have been used in in-vitro cultures for the production of interferon and monoclonal antibodies (*38*). This approach has been shown to have many advantages over standard cell culture techniques (*38*). Bacteria have also been microencapsulated within artificial cells (*53*). This way, the bacterial enzymes continue to take part in the sequential multistep enzyme system required for substrate conversion and possible applications in biotechnology.

Other biologically active materials, such as hormones, antigens, antibodies, and vaccines, have also been enclosed within artificial cells (*5, 6, 21*).

BLOOD SUBSTITUTES

The shortage of blood donors, the transmission of hepatitis and AIDS, requirements for cross-matching, and short durations of storage are some of the problems associated with the use of donor blood in transfusion. The two systems being investigated as possible red blood cell substitutes at present are derived either from hemoglobin or from organic materials (*54–57*).

Research was initiated here as early as 1956 to investigate the feasibility of hemoglobin artificial cells (*1–5*). Since, then, extensive studies have been carried out in a number of centers to study the preparation of different types of artificial hemoglobin cells. These studies might be divided into two major approaches (Fig. 4). One approach is to microencapsulate hemoglobin solution with different types of spherica l ultrathin membranes. Collodion, nylon polystyrene, heparin-complexed membranes, cellulose acetate, and other synthetic polymers have been studied (*1–5, 8, 54*). Lipids alone or complexed with protein or polymer have also been used to form spherical membranes (*14–16, 58*). A second major approach is the crosslinking of hemoglobin by one of two ways (*2–5, 29, 54*). Microdroplets of hemoglobin can be crosslinked at the surface to form an ultrathin crosslinked hemoglobin membrane (*2–5, 54*). All of the hemoglobin in the hemoglobin microdroplet can also be crosslinked to form a crosslinked hemoglobin complex (*2–5, 29*). The size of each crosslinked hemoglobin complex depends on the total number of hemoglobin molecules (*54*). During this process, intramolecular crosslinking also takes place, thereby stabilizing the tetramer form of hemoglobin. Intramolecular crosslinking of the hemoglobin molecule without crosslinking

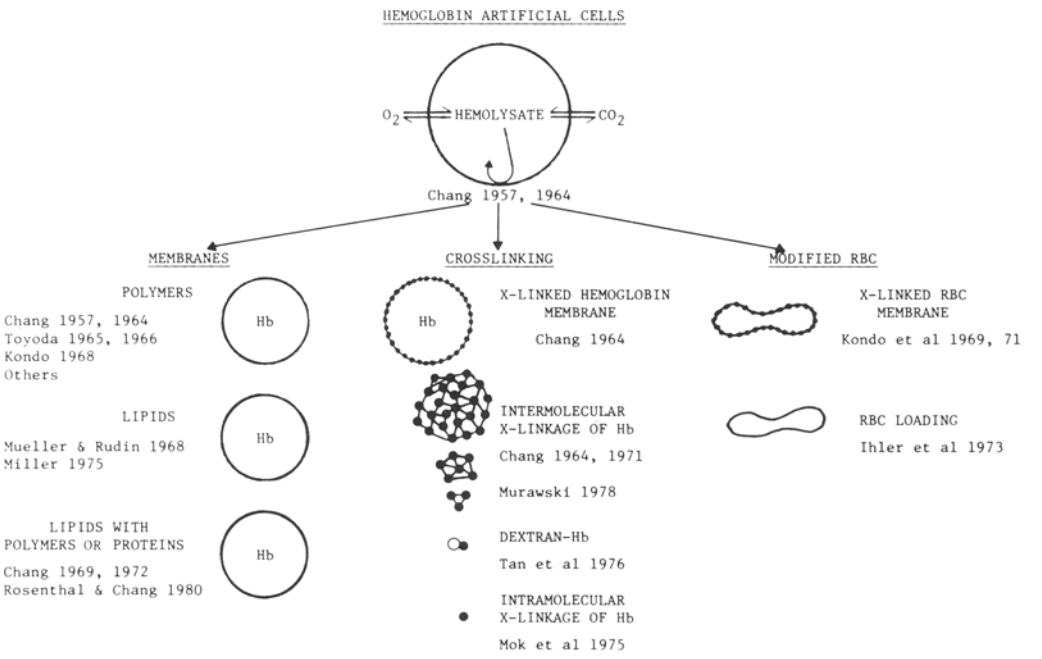

Fig. 4. Development of artificial red blood cell substitute based on hemoglobin (*54*).

has also been carried out to prevent the conversion of tetramers into dimers (*59*). Each hemoglobin molecule can be covalently linked to a molecule of dextran to create a large molecule that is not as rapidly excreted by the kidney (*60*). The original problem related to artificial hemoglobin cells is that, when injected intravenously, they are rapidly removed by the reticuloendothelial system (*2–5*). Progress is being made in this area using less crosslinked hemoglobin complexes, polyhemoglobin (*54, 57, 61*), smaller lipid membranes artificial cells (*58*), or a small crosslinked hemoglobin membrane (*62*). Our studies show that crosslinked artificial hemoglobin cells, each consisting of soluble polyhemoglobin, survive much longer in the circulation when compared to free hemoglobin (*61, 63*). Extensive research is being carried out to develop this crosslinked hemoglobin approach for possible clinical testing (*57*).

Artificial red blood cell substitutes have also been prepared based on organic materials. Since silicone rubber is excellent for oxygen transport, silicone rubber microspheres, consisting of two parts of silicone rubber and one part of hemolyzate, were first prepared (*10*). This type of silicone rubber microsphere was found to carry oxygen well (*10*). However, since they were solid, silicone rubber microspheres did not survive well in the circulation. At about the same time, another group found that silicone oil and fluorocarbon oil also work well in O_2 carriage (*64*). Unfortunately organic liquids as such cannot be infused into the circulation as blood substitutes. The next step was the use of a fine emulsions of fluorocarbon

(*64–66*). These fine fluorocarbon emulsion were effective in O_2 carriage when tested in rats. However, there were problems related to blood compatibility and emulsion stability. These problems were solved by using a modification of our approach of using albumin coating (*5, 12*) or lipid coating (*5, 15, 16*) on these microdroplets to form an "artificial cell membrane" (*56*).

The present status of the hemoglobin-based artificial cells and fluorocarbon-based artificial cell is as follows. Artificial fluorocarbon cells are the first type of artificial blood substitute to be ready for clinical trial. The results obtained so far have been exciting (*55–57*). However, the major problems related to artificial fluorocarbon cells are: (1) their short survival in the circulation; (2) the accumulation of fluorocarbon in the body; and (3) the requirement of oxygen concentrations much higher than that present in the atmosphere. The hemoglobin-based blood substitutes are still in the stage of being developed for clinical trial. One of the advantages of artificial hemoglobin cells is the biodegradability of the hemoglobin component. It will, therefore, solve the problem of accumulation in the body. Furthermore, it can function with the normal concentration of oxygen found in the atmosphere.

BLOOD DETOXIFIER (FIG. 5)

Artificial cells containing adsorbents and other detoxifying agents have been developed here for the construction of blood detoxifiers (*5, 10, 12, 67, 68*). For example, the application of a 0.05 μm ultrathin membrane coating on each charcoal granule prevents release of charcoal powder emboli and makes the surface blood compatible. At the same time, toxins, drugs, and waste metabolites can equilibrate rapidly across the ultrathin membrane to be removed by the activated charcoal. Charcoal granules with a 0.05 μm thick collodion membrane coating is sufficiently blood compatible for most clinical applications. However, blood compatibility can be further increased when required (e.g., for severe liver failure) by the adsorption of albumin to the collodion membrane (*12, 67, 69*). The albumin coating also acts as a facilitated carrier mechanism for loosely protein-bound substances in the bloodstream (*70*). These artificial cells containing activated charcoal (100–300 g) are placed in a container with screens on both ends. These screens retain the artificial cells, while allowing blood to perfuse through to come in direct contact with the artificial cells. Dogs that had received lethal doses of barbiturate, salicylate, and Doridan, when treated with this blood detoxifier, recovered rapidly as the drugs were rapidly removed from the circulation (*5, 12*). This led us to apply the technique in patients for the treatment of acute drug intoxication (*71, 72*). The patients recovered rapidly as the drugs were rapidly removed (*71, 72*). The artificial cells are much more effective than the large and bulky standard hemodialyzer in removing drugs like Doridan,

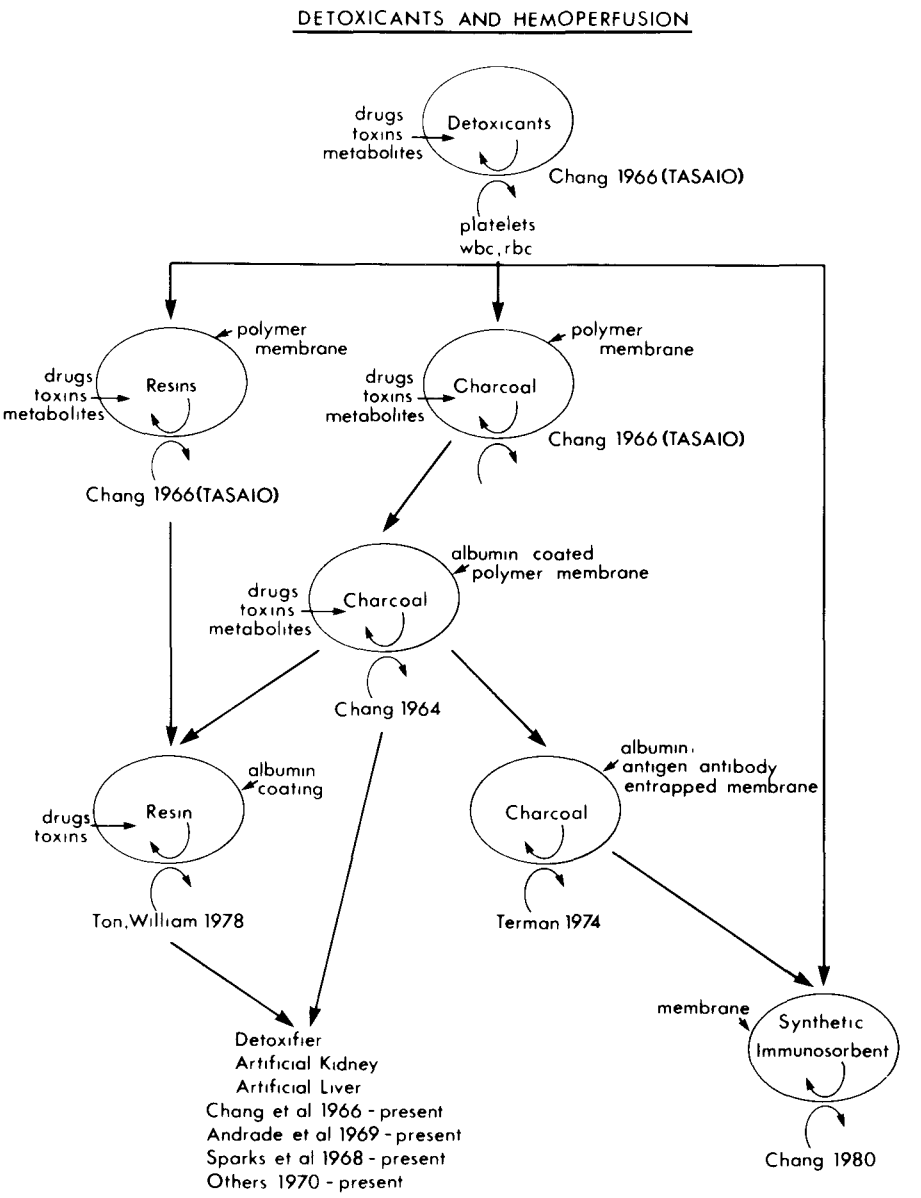

Fig. 5. Development of artificial cells for use in detoxification and immunosorbents (*95*).

Methyprylon, and methaqualone. Clearances for the blood detoxifier were up to 230 mL/min, compared to clearances of less than 60 mL/min using standard hemodialyzers. This led us and others to treat more patients (*68, 73–82*). The use of artificial cells containing activated charcoal initiated here is now a routine procedure in most medical centers around the world for the treatment of patients with drug poisoning involving drugs with a small volume distribution (*73–82*).

The results described stimulated a number of other centers and industries to develop blood detoxifiers based on artificial cells (*73–82*). In applying polymer membranes to activated charcoal, the thickness, permeability and the blood compatibility of the membranes are extremely important factors. As a result, the presently available commercial systems based on this approach vary somewhat in blood compatibility and efficiency.

Ion exchange resins when microencapsulated within artificial cells cannot adversely affect blood cells, but retain their ability to remove permeant toxic substances (*5, 10*). Another approach is to apply the albumin coating technique developed here (*5, 12, 67, 69*) to resins like amberlites (*83, 84*). This way, amberlites can now be used for blood detoxification without adverse effects on blood cells.

ARTIFICIAL LIVER

There is at present no complete artificial liver support system available. The first success with an artificial liver support system was demonstrated here in 1972 (*85, 86*). Treatment with the artificial cell blood detoxifier resulted in the repeated temporary recovery of full consciousness in unresponsive grade IV hepatic coma patients (*85, 86*). These results stimulated other centers to use this approach for patients with hepatic coma. A total of about 500 cases have been treated around the world and about 60% of the patients treated regained consciousness (*81–91*). However, the recoveries of grade IV hepatic coma patients were only temporary, and no significant improvement in long-term survival rate could be demonstrated. As a result, further research must still be carried out.

A study carried out here using the galactosamine-induced fulminant hepatic failure (FHF) rat model has demonstrated that the artificial cell detoxifier significantly increased the long-term survival rates of FHF rats (from 30% in control, to 70% in treated) (*92*). However, we also showed that this increase in survival rate was possible only when the FHF rats were treated in the earlier stages of hepatic coma, but not in the later stage of coma when irreversible damage may have already occurred (*93–95*). These results have prompted another center to treat patients in the earlier stages of coma (*96*). They showed that treatment of grade III FHF coma patients resulted in improved survival rates (30% in control, to 70% in treated); while treatment of grade IV FHF coma patients did not improve the survival rate (*96*). Thus, the timing of treatment is very important in using the blood detoxifier. This first successful artificial liver support system should stimulate further studies for a more complete artificial liver support system for the later stage of coma and also for chronic terminal liver failure patients (*97*). For instance artificial cells containing

enzyme systems are being studied for carrying out some metabolic functions of the liver. Ammonia, tyrosine and some other amino acids found elevated in liver failure may contribute to hepatic coma. We have prepared artificial cells containing tyrosinase (*51*). When retained in extracorporeal chambers perfused by blood, they effectively lower the systemic tyrosine levels in the blood of FHF rats (*51, 52*). We are also studying the use of artificial cells containing multienzyme systems with cofactor recycling for the sequential conversion of ammonia into different amino acids (*40, 42, 43*).

ARTIFICIAL KIDNEY

Artificial cells have been used here as the basis of an artificial kidney to replace the bulky and expensive hemodialysis machine. Initial studies in patients showed that artificial cells containing activated charcoal can maintain terminal renal failure patients alive and eliminate their uremic symptoms of nausea, vomiting, fatigue, bleeding, and other problems (*98–100*). This can be accomplished by using the blood detoxifier for 2 h, instead of the 8-h standard hemodialysis (*98–100*). However, the blood detoxifier based on artificial cells does not remove water, electrolytes, or urea. To solve this problem, the detoxifier can be used in series with the hemodialysis machine, which is used in this case mainly to remove water, electrolytes, and urea (*101*). With this combination, the time or treatment could be cut down to 2 h (*101*), resulting in the patient having more time for work and other responsibilities. Furthermore, this combination procedure would reduce the cost of space and staff requirements for hospitals. Both this approach and our earlier results indicating that the artificial cell detoxifier can eliminate uremic complications (e.g., nausea, vomiting, peripheral neuropathy, etc.) have been supported by further studies here and in other centers (*68, 70, 73, 80, 82, 90, 91, 101–107*). A recent development involves the combination of this artificial cell detoxifier in series with a capillary membrane system into one single unit to simplify the approach even further (*108–110*).

Despite the decrease in time of treatment with the combined system, the hemodialysis machine is still required to remove water, salt, and urea. Studies have been carried out here to determine how to eliminate the need for the expensive and bulky hemodialysis machine. The artificial cell blood detoxifier was combined in series with a small ultrafiltrator (*101, 111*). This way the artificial cells remove the uremic waste metabolites and toxins while the ultrafiltrator removes sodium chloride and water. This way the hemodialysis machine is no longer required and the smallest artificial kidney based on artificial cells is now available. This has been tested successfully on a long-term basis in patients (*112*). Potassium could be removed by the oral administration of potassium adsorbent.

Means for phosphate removal and urea removal are being studied to complete this system.

Hemoperfusion with artificial urease cells in dogs can lower the system urea level to 50% within 45 min (*10*). The ammonia produced can be removed by microencapsulated ammonia adsorbent (*10*). However, the ammonium adsorbents available at present do not have sufficient adsorbing capacity to allow their clinical application. Another approach we tested was to administer artificial urease cells and ammonia adsorbent into the intestinal tract (*5, 24, 44*). This way, urea was converted into ammonia, which is removed by ammonia adsorbent. This was extended further by other laboratories (*25, 49*) and is now being tested clinically (*50*). Artificial urease cells with a liquid membrane that is permeable to urea, but impermeable to ammonium ions, have also been investigated (*11*). We are studying another approach using artificial cells containing a multienzyme system for the conversion of urea into ammonia, which is then sequentially converted into different types of amino acids (*17, 33, 40, 42, 43*). In the meantime, oxystarch has been modified into a more acceptable form for oral administration for the removal of urea (*113*). The problem with oxystarch is the large amount required. While waiting for the development of an optimal urea removal system, preliminary studies showed that it is possible to substitute one of the three weekly hemodialysis treatments on a long-term basis (*112*). With this approach, the hemodialysis machine and dialyzate fluid are not required. This is the simplest and most compact artificial kidney available.

IMMUNOSORBENT (FIG. 5)

Albumin has been adsorbed on the surface of collodion artificial cells to make the surface blood compatible (*5, 12, 67, 69*). This albumin coating also interacts with different substances in the blood (*70*). It was found that albumin on the surface of the ACAC could also be used to remove antibodies to albumin in the circulating blood of animals (*114*). This has led to the incorporation of antibodies or antigens onto the collodion coated charcoal for the removal of specific antibody or antigen from the circulating blood (*114*). For example, protein A on the collodion-activated charcoal can interact with plasma that was found to initiate immunological reaction to stimulate the rejection of breast cancer in patients (*115*). However, these results are preliminary (*116*).

Synthetic immunosorbents that remove antibodies to blood groups A and B have been treated with albumin or an albumin collodion coating result in a blood compatible system that does not release emboli (*117*). This has been applied in an initial clinical trial to remove blood group antibodies before bone marrow transplanation (*118*).

MAGNETIC MICROCAPSULES

Originally we prepared artificial cells containing magentic material in order to retain them at a specific location or to direct their movement and locations (*10*). For instance, artificial nylon membrane cells containing enzyme, protein, and magnetic material were prepared. They were placed in an extracorporeal chamber through which blood circulated. By applying a magnetic field external to the chamber as blood circulates through, we were able to retain the magnetic microcapsules in specific regions of the chamber and by stirring with the magnetic stirrer the microcapsules could also be stirred (*10*). This principle has stimulated recent interests for targeting of magnetic microcapsules in the circulation. Thus magnetic microcapsules containing drugs, chemotherapeutic agents, or radioactive substances have been prepared for injection to selectively retain them at specific sites in the circulation of experimental animals (*30*). Though further development needs to be carried out, the general principle may find interesting applications not only in medicine, but also in various types of biochemical reactor systems.

THE ARTIFICIAL CELL AS A DRUG DELIVERY SYSTEM

The use of artificial cells containing an enzyme for experimental therapy (*2–6*) is a form of drug delivery system. The details of this have already been discussed under the earlier section. The same principle described could be also applied to systems for medications. Detailed reports in this area will be made by a number of contributors to this volume. One of the very popular areas involving artificial cells is the use of lipid-membrane artificial cells that include liposomes. The original approach was to use liposomes to encapsulate drug (*18, 19*). However, in this form the amount of the carrier material is much higher than the drug being carried. The present approach is a return to the system closer to the original artificial cells of a spherical ultrathin membrane of lipid material (*14–16, 46*). In this form, by making the artificial lipid cell very small, the lipid membrane can be stabilized, and much larger amounts of drug can be carried. The area has been reviewed in detail elsewhere (*46*). The other approach, which is quite popular at present, is the use of biodegradable synthetic polymer. For instance, polylactic acid has been used to microencapsulate hormones, vaccines, and other biological materials (*21, 35*). When prepared using insulin granules, the insulin can be released at different rates depending on the mode of preparation (*21*). This approach is also being used by a number of other groups for the slow release of therapeutic agents, especially different types of hormones. A detailed review is available (*119*). Another biodegradable material based on our earlier approach of crosslinked protein microcapsules

(*2–6,29*) has now also been developed by various groups as biodegradable drug carrier systems. Another interesting approach in the use of nanocapsules or microparticles has been reviewed elsewhere (*120*).

OTHER AREAS

The uses of artificial cells in other areas of medicine and biotechnology have also been studied. One of these uses is the clinical laboratory. Hormones in the blood are usually partly bound to protein, and in some clinical situations, it is important to know their free and protein-bound fractions. However, it is rather laborious to do this type of analysis. Recently, artificial cells were prepared by interfacial polymerization to contain isotope-labeled thyroxine and antibodies to thyroxine. By suspending these artificial cells in serum, free thryoxine in the serum can diffuse across the artificial cell to displace the radioisotope-labeled thyroxine bound to antibodies. By analyzing the radioactivity, a protein-free fraction of thyroxine can be analyzed (*37, 38*). This approach has also been applied to other types of hormones (*38*). Another application is the use of artificial cell containing a nutrient required for aquatic culture. This was originally done by Jones (*121*) using the technique of interfacial polymerization to prepare the required microcapsules. Such microcapsules can be filtered, ruptured, and digested. Artificial cells containing activated charcoal in the presence of desferrioxamine can effectively remove trace metals (e.g., aluminum) from the blood of patients (*122*).

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