ARTIFICIAL CELLS

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ABSTRACT

Artificial Cell research carried out by Chang has led to development and approval for routine clinical uses in a number of areas:

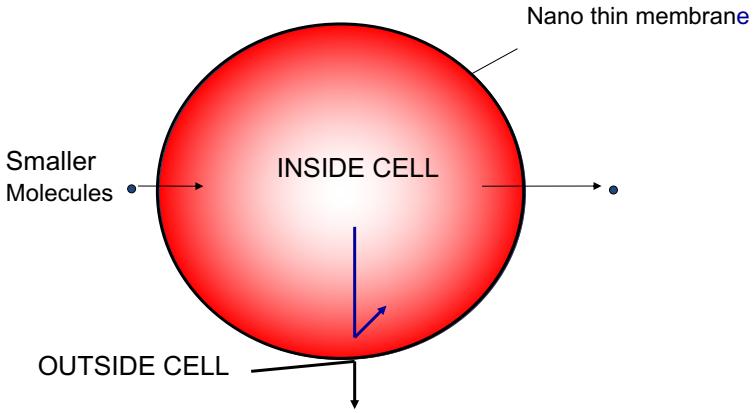
- For use in COVID_19 vaccines.
- Hemoperfusion for COVID-19 cytokine storm treating poisoning, partial support of liver and renal failure, and for some immunological diseases.
- For use as first-generation blood substitute in countries with HIV contaminated donor blood.
- As a number of drug delivery systems.
- PEG-asparaginase for use in leukemia treatment.
- Recently approved as PEG-Phenylalanine ammonia lyase for the treatment of adult PKU.

This is just the beginning of the actual routine clinical use of artificial cells since the principle of artificial cell is just beginning to be actively explored into other areas of nanomedcine, biotherapeutics, blood substitutes, targeted drug delivery, enzyme/gene therapy, cancer therapy, cell/stem cell therapy, nanoparticles, liposomes, bioencapsulation, replicating synthetic cells, cell encapsulation, biosorbent/immunosorbent hemoperfusion/plasmapheresis, regenerative medicine, encapsulated microbe, nanobiotechnology, nanotechnology and other areas. More futuristic research includes nanorobot, nanocomputer, multimodal locomotion delivery robot and others.

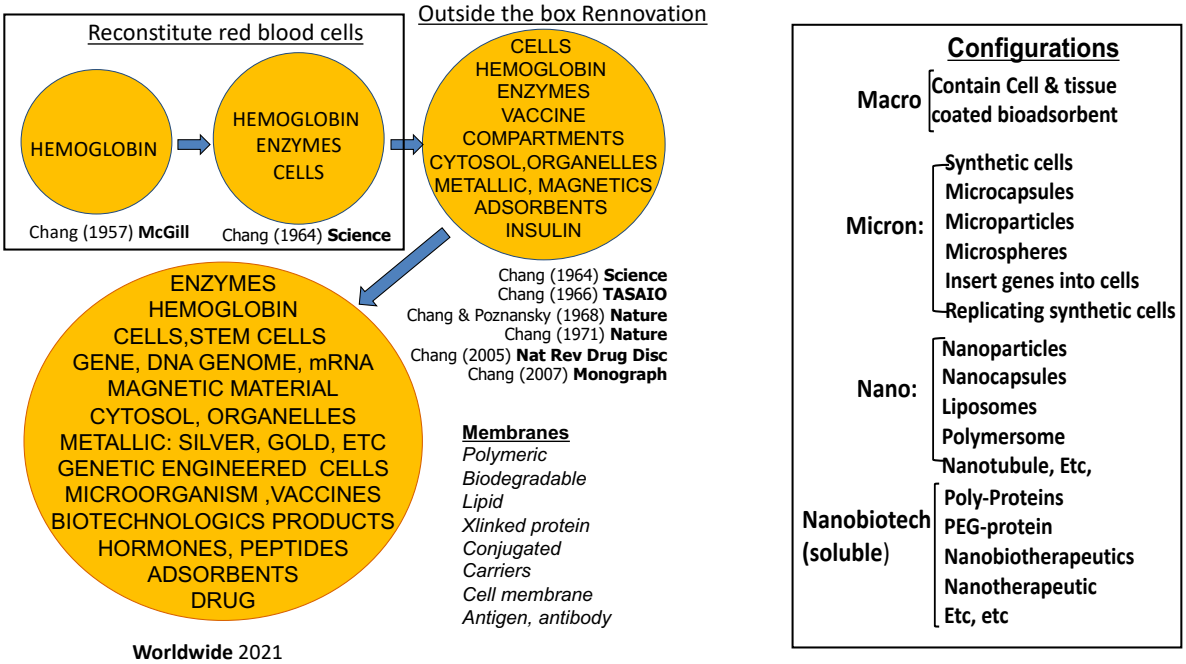
IDEA OF ARTIFICIAL CELLS

Basic Principle of Artificial Cells

(Chang 1957 McGill, 1964 Science)



The very first humble "artificial cells" reported by Chang in 1957 (1,2) is not to reproduce biological cells, but to use available basic knowledge to prepare very simple system for possible uses in medicine and other areas (Fig. 1). This author predicted in his 1972 monograph on Artificial Cells (6) that *"Artificial Cell is not a specific physical entity. It is an idea involving the preparation of artificial structures of cellular dimensions for possible replacement or supplement of deficient cell functions. It is clear that different approaches can be used to demonstrate this idea*". There are unlimited possibilities in variations for the artificial cell membranes and contents (Fig. 2). Artificial cells can be of macro, micro, nano and molecular dimensions (Fig. 2). Each of these has unlimited variations in configurations. Each



configuration resulted in a new terminology with many arbitrary subdivisions of "artificial cells" under the guise of different names. All these mean that there are many areas of application in medicine and even outside medicine (Table I)

ARTIFICIAL CELLS: APPLICATIONS (2019)

Microdevice and nanodevice
Drug delivery:
Blood Substitutes and oxygen therapeutics
Biotherapeutics, Immunotherapeutics:
Enzyme and gene therapy:
Cell & Stem Cell Therapy:
Biotechnology & Nanobiotechnology
Nanomedicine
Regenerative medicine
Agriculture, Industry, Aquatic culture
Nanocomputers and nanorobatics
Nanosensors
Replicating synthetic cells etc
Other transformative possibilities

It is only in the last 20 years that many of the original ideas on artificial cells are being increasingly applied and extended by researchers around the world. This is because many of the original ideas (2-7) were reported years before the modern era of nanotechnology, regenerative medicine, blood substitutes, biotechnology, gene therapy, stem cell therapy, cell therapy and other areas. Thus, following his 2005 review on "therapeutic applications of polymeric artificial cells" in Nature Review Drug Discovery (8), a timeline prepared by the editor shows that Chang has made 20 of the 23 major discoveries in related areas up to that time. However, since that time, other groups are making rapid and exiting progress and numerous discoveries. Each major progress in other areas has led to stepwise progress in artificial cells. First there is the coming of age of polymer chemistry and biomaterial. Then there is the recognition of the importance and developments in biotechnology. Then there is the progress in molecular biology and genomics. All these has contributed to a quantum leap in the area of artificial cells. One can expect that there will be important future progress in other areas, for example, artificial intelligence and nanorobots, that will contribute to unlimited progress by increasing number of groups world-wide in the area of artificial cells. We have only touched the surface of the potential of the extension, innovations and uses of artificial cells (Fig. 1-3). Space only allows for a general overview follows by some examples of the different configurations and their applications. More details are available elsewhere (12).

HISTORY

In 1957, while a final year honours B.Sc. Physiology student at McGill University, I came up with the idea of preparing artificial cells. I thought that since cells are the fundamental units of all organs and tissues, artificial cells should have implications for many areas of medical uses. I went around talking to students about this and even to chemistry professors thinking that they would have methods for doing this. They all gave me a funny look and the chemists even told me that this is impossible. So, I went to my dormitory room and started to try different ways to do this. After many unsuccessful attempts. I finally came up with a very preliminary method. All final year honours Physiology students have to complete an assigned research project. I gathered up my courage and went to see a young Professor Burgen who was in charge of the honours program (He shortly returned to U.K. to become Sir Arnold Burgen, FRS). He sent me the following for my 60th anniversary of the invention of artificial cells: *"……..I still recall you coming to see me and saying that you would prefer to do a different project and would like to try to make artificial red blood cells. I think I said go ahead without much expectation. The start of a life of a very successful career in science! ……..,"* With beginner's luck, I was able to prepare artificial red blood cells that has some of the oxygen carrying properties of red blood cells. The department asked for a sample for one of the professors to check and he also obtained the same type of oxygen dissociation curve. As a result, the honours thesis was approved as "Chang, T.M.S. (1957) Hemoglobin corpuscles. Report of a research project for Honours Physiology
http://www.medicine.mcgill.ca/artcell/514.pdf

However, the department chairman did not want me to publish this rather outlandish idea. So I continued this research while finishing my medical school. After this I wanted to continue with PhD research, but the chairman was not too receptive since there was no one doing this research to direct me. After much discussion he kindly organized a PhD committee consisting of senior full professors all holding FRS: Physiology, Biochemist, Anatomy and Chemistry. Midway through my PhD the chairman with the agreement of the committee finally agreed to let me submit a paper for publication under the condition that it should not be called artificial cells and I should be the sole author. Surprisingly Science accepted it (2) (Chang 1964). After my PhD, the chairman did not have a position for this type of research. The associate dean, Professor Bates, who later became the chairman, helped me to apply for MRC career investigator awards (junior followed by senior). With research progress leading to clinical trials (Chang & Poznanski Nature 1968, Chang Nature 1971 and others), I rose to tenured full professor in 6 years in the departments of Physiology and Medicine and later Biomedical Engineering. One advantage of being a tenured full professor is that I was able to publish an invited monograph on "Artificial Cells" in 1972 (6)

BASIC METHODS

This review cannot cover all the important methods of the preparation of the numerous configurations of of artificial cells. Instead, we shall first look at the historical basic approaches to be followed later in more details using specific examples.

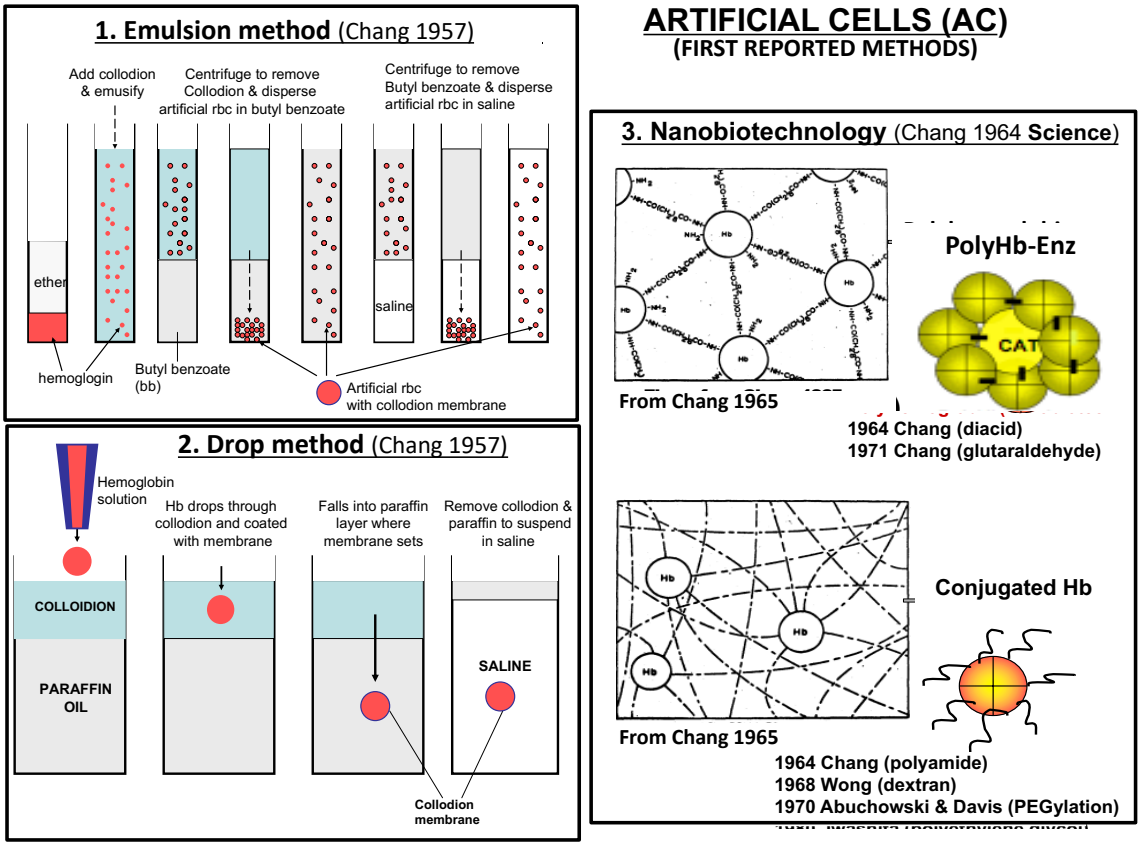


Fig.. Upper left: *Original (1)(Chang 1957) emulsion method of preparing micro-dimension artificial cells. Since extended to physical or chemical methods for microscopic and nanodimension artificial cells.* ***Lower left:****. Original (1)(Chang 1957) drop method for the preparation of large artificial cells. This has been now been extended and modified for cell/stem cell encapsulation.****lower left:*** *Basic method (Chang 1964 Science)(2,7) of bifunctional agents to assemble and crosslink hemoglobin (Hb) into PolyHb that has evolved into the preparation of soluble polyhemoglobin and other biotherapeutics.****Lower middle*** *: Basic method of conjugating hemoglobin to polymer (1)(Chang 1964 Science).that has evolved into the use of other polymers like the Pegylation (PEG-protein) Updated from Chang (8. 9, 12)*

Micro and nano dimension: The basic principle is to use emulsion followed by the use physical or chemical methods to form membrane around each micro droplet (1-2). The diameter is determined by the diameter of the emulsified micro or nano dimension droplets. Extensive novel emuslsion methods developed around the world are now available for use. This principle has since been extended using modified physical or chemical methods for the preparation of microscopic or nanodimension artificial cells that are also called microcapsules, nanocapsules, liposomes, microparticles, nanoparticles, polymersomes, etc. Microfluidizer is a new way of preparing artificial cells (10a)

Macro dimension: The drop method for the preparation of large artificial cells (1) has now been extended and modified using modified physical or chemical methods for cell/stem cell/tissue encapsulation. This will be described in more details later.

Crosslinking of proteins. The original basic method (2,7) of the use of bifunctional agents to assemble and crosslink hemoglobin (Hb) into PolyHemoglobin. has been extended into many other areas of nanobiotechnology and nanobiotherapeutics. This will be described in more details later.

Conjugation of protein. The original basic method of conjugating hemoglobin to polymer (2) has evolved into the conjugation of hemoglobin to soluble dextran or soluble (PEG) polyethylene glycol. Pegylation of proteins (PEG-protein) is now a popular approach in biotherapeutics (10)

EXAMPLES OF ROUTES OF ADMINISTRATION

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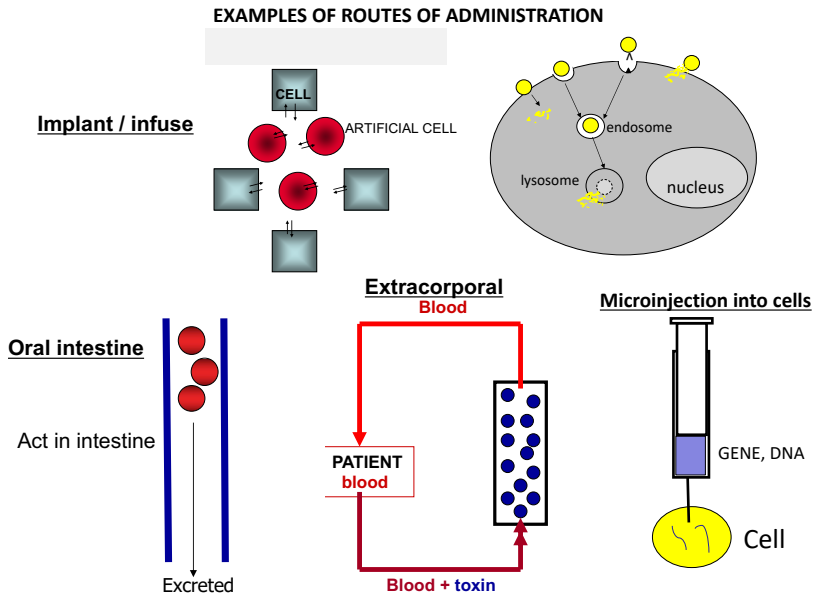


Figure contains examples of possible routes of administration for the function of artificial cells in the body. Generally speaking, regulatory agencies are less worry about the use of artificial cells that are not implanted or injected into the body. We therefore started with artificial cells that are not implanted but act in a device for the extracorporeal route. This has resulted in the early approval of the use of artificial cells in patients way back in 1980. This is in the form of a hemoperfusion device.

ARTIFICIAL CELL BASED HEMOPERFUSION
Including recent use to treat COVID_19 patients with cytokine storm

Basic principles

It is common knowledge that for the same volume of particles the smaller the particles, the larger would be the total surface area. It is also known that the theoretical diffusion across a membrane is proportional to the total surface area and inversely proportional to its membrane thickness. However, my 1966 analysis of the implication of combining all these factors for artificial cells of micro dimension is way beyond expectation (5). Figure shows an updated analysis (11) of the theoretical mass transfer of a fixed volume of 0.01 um membrane thickness artificial cells with different diameters. This is compared to an artificial kidney (hemodialysis) machine with a mass transfer of 1. The mass transfer increases with decreasing diameter of artificial cells so that at the micro diameter range it can increase to 100 times that of an artificial kidney. At the nano diameter range, this can increase to an amazing 1,000 times above that of an artificial kidney. Thus, artificial cells of different diameter containing different bioactive material can become efficient micro/nano dialyser/bioreactor with unlimited possibilities (Fig)

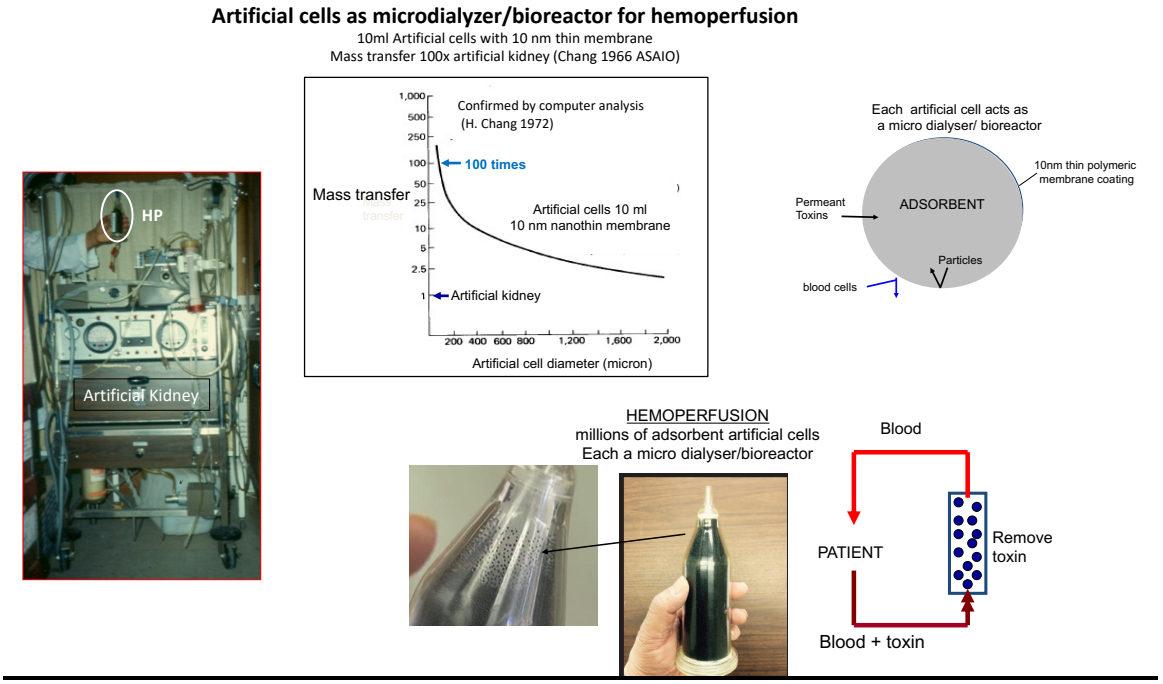


Fig. ***Center:*** *Theoretical mass transfer of 5ml 0.01 um membrane thickness artificial cells with different diameters. This is compared an artificial kidney machine with a mass transfer of 1.*
Upper right*: Thus, artificial cells containing bioactive material can become efficient micro/nano dialyser/bioreactor.* **Lower:** *70 grams 90 micron diameter adsorbent artificial cells retained inside a small container by screens at either end.* ***Left:*** *Its small size is compared to an artificial kidney. Updated from Chang (5,6,8,9, 11)*

Based on this analysis, 70 grams of 90 micron diameter adsorbent artificial cells are retained inside a small container by screens at either end. The sorbent artificial cells remove toxins or drugs from the blood of patients perfusing through the column. The membrane of the artificial cells prevents the adsorbent from being released into the body and also prevents the adsorbent from damaging the blood cells (Fig. 6).

This result in a cup size miniaturized hemoperfusion device with hundred times the efficiency of a hemodialysis (artificial kidney), the size of a washing machine (Fig).

Clinical use in patients

The author starts study on the use of artificial cells containing adsorbents for hemoperfusion. This included personally carried out scaled up, animal testing and clinical trial in patients. He shows the safety and effectiveness for using this first in animals then in patients. Figure shows the result of one of the many patients the author has carried out (13). This is a suicidal patient who ingests 3 times the lethal dose of a sleep pill, methryprylon. Five hours of standard hemodialysis treatment cannot lower the drug level and the patient remains comatose, hypotensive with cardiac arrests. When the author starts hemoperfusion treatment the plasma methryprylon level decreases rapidly in 2 hours and the patient is no longer comatose nor hypotensive and shortly recovers completely.

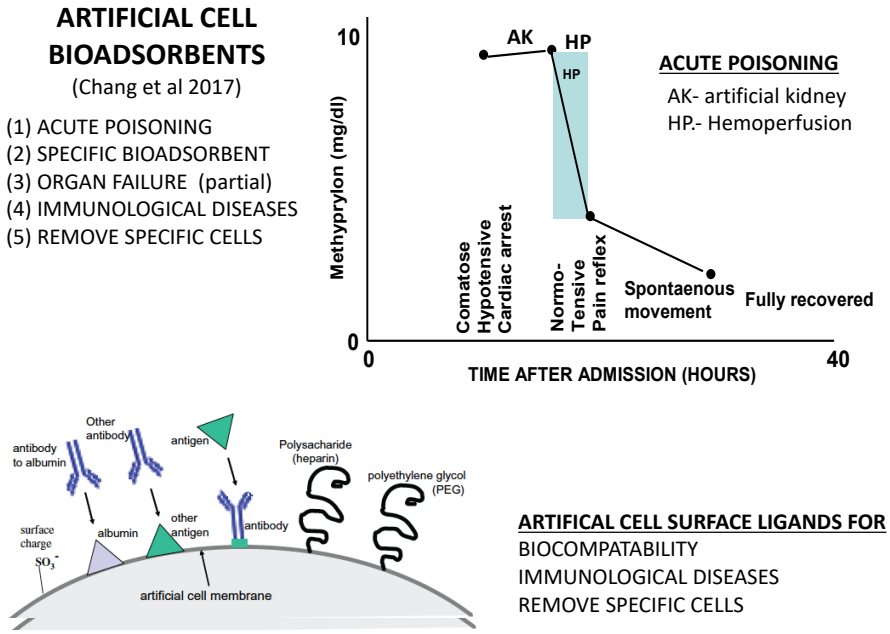
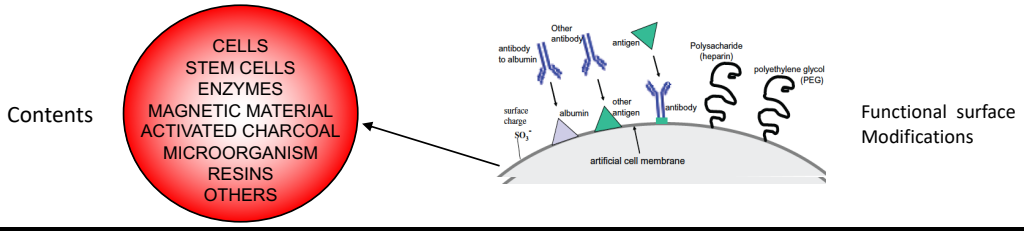


Fig. Left *Present status of clinical uses of Hemoperfusion (from Chang et al , 2017 Book on hemoperfusion).* ***Right****: Example of a sleeping pill overdose suicidal patient (Chang et al 1973). Standard dialysis (AK) is not effective but hemoperfusion (HP) quickly lowered the plasma level and rapid recovery.. Updated from Chang (9, 11)*

Following this first case, similar results are obtained in a number of other patients (13). He has also showed its effectiveness as partial support in patients for kidney failure and liver failure to remove toxic molecules. These results have led to FDA approval for routine clinical uses. Hemoperfusion is now an accepted routine clinical use for the treatment of patients with suicidal or accidental overdose of some medications around the world. A 2017 book (11) by specialists around the world shows that approach is being used extensively around the world, especially in countries where these can be manufactured with affordable costs.

Possible extensions

Possible variations: present and future: (From Chang et al 2017)



What is exciting is that extensive modifications and extension into many other uses including the use of specific bioadsorbents, immunosorbents. Furthermore, surface properties of artificial cell membranes can be varied by (1) incorporation of negative or positive charge; (2) incorporation of albumin to increase blood compatibility; (3) incorporation of antigens to bind antibodies or antibodies to bind antigen; (4) incorporation of polysaccharides like heparin or polyethylene glycol (PEG) to increase biocompatibility (9-11, 14-19) (Fig. 7). This has led to systems for the specific removal of endotoxins, for the treatment of immunological diseases like Lupus and for the removal of unwanted cells. This is now such a large area with numerous publications that please refer to the book for more details (12).

Hemoperfusion for COVID_19 patients

Patients with very severe COVID-19 may die despite ventilator and oxygenator support due to a sudden and massive increase in certain toxic factors (inflammatory factors, cytokines storm). These can damage organs resulting in multi-organ failure and death. Artificial Cell hemoperfusion has earlier been shown to be effective for the lowering of systemic cytokine in a number of medical conditions. A Chinese company produces a similar device that shows efficacy in the treatment of severe COVID_19 patients (2020 Lancet preprint (20): Survival 47 (62%) in HP and 15 (38%) in the control group ($p<0.05$).

Hemoperfusion in severe COVID-19 cytokine storm (from Zhou et al 2020 Lancet online)
Total 47 patients. 26/47（55%）Treated with hemoperfusion. 21/47(45%) Standard treatment
72 hours after Hemoperfusion group: decrease in serum cytokines, Improvement in oxygen uptake
% Deaths on day 28 Hemoperfusion group: 38% Standard Px: 62% $p < 0.05$)
Intensive Unit (ICU）-free days Hemoperfusion group: 6.5 days Standard Px: 2.0 days。 $p < 0{\cdot}01$)

Authorized medical devices for uses related to COVID-19: List of authorized medical devices other than testing devices

IO authorization date	IO authorization ID	Manufacturer	Device name	Device identifier	Device first issue date	Device type
2021-05-04	317573	Jafron Biomedical Co., Ltd. (China)	Disposable Hemoperfusion Cartridge	HA330	2021-05-04	Patient Care Technologies And Accessories - Blood Purification System
			Disposable Hemoperfusion Cartridge	HA380	2021-05-04	

This is now used in Europe and Asia.. Health Canada Regulatory agency corresponded with the Chinese company for further details and after 9 months of careful analysis it has **approved this as an emergency treatment for COVID_19 in Canada**

BLOOD SUBSTITUTES

Unlike the use of artificial cells in a hemoperfusion device that is outside the body, this is an example where large volumes artificial cells have to be infused intravenously into the body. Thus, even though this is a very important and urgent life saving method, it needs more time before regulatory approval.

Why blood substitutes?

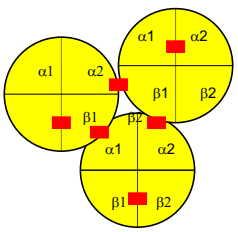
. Under normal circumstances, donor blood (rbc) is the best replacement for blood. HOWEVER:

- Natural epidemics (e.g. HIV, Ebola, COVID-19,etc) or man-made epidemics (terrorism, war, etc) can result in contaminated donor blood or disqualified disease contact donors. Unlike rbc, blood substitutes can be sterilized.
- Heart attack and stroke are usually caused by obstruction of arterial blood vessels. Unlike rbc particles, blood substitute is a solution and in animal studies it can more easily perfuse through obstructed vessels to reach the heart and brain.
- Severe blood loss from accidents, disasters or war may require urgent blood transfusion that cannot wait for transportation to the hospital for blood group testing. Unlike rbc, blood substitutes do not have blood groups and can be given on the spot (Fig. 8).
- Red blood cells have to be stored in refrigeration for up to 42 days thus difficult to transport and store in disaster and frontline. Blood substitutes can be stored at room temperature for more than 1 year, compared to rbc of 1 day at room temperature.
- In very severe hemorrhagic shock there is usually a safety window of 60 min for blood replacement, beyond which there could be problems related to irreversible shock. Animal study shows that one type of blood substitutes with enhanced rbc enzymes can prolong the time.

What is the present status around the world?

After the first report of artificial red blood cells in 1964 (2) people felt that blood substitute is a simple matter that could be quickly developed when needed. Thus blood substitute research was put aside and only the other areas of artificial cells were extensively developed around the world for other wide spread uses. When AIDS arrived in 1989 there was no blood substitutes and many patients were infected with H.I.V. contaminated donor blood. It is only then that intense R&D on blood substitutes was belatedly carried out around the world (20-35). It was found out too late that blood substitute requires the same long-term research as in any other medical research for cancer and other diseases. Thus, the present status is as follows (Fig.):

(1) First generation: Oxygen carriers (HBOCs):



Polyhemoglobin
Diacid (Chang 1964)
Glutaraldhye (Chang 1972)

FIRST GENERATION BLOOD SUBSTITUTE

No initial interest until H.I.V. contaminated donor blood crisis in the 1980s

Then Biopure Co prepare their glutamer-250 product , based on the 1972 published method of Chang using glutaraldehye crosslinked polyhemoglobin.

Clinical trials (24) result led to **South Africa and Russia approve the use of this product to avoid H.I.V. contaminated donor blood**. This is based on risk/benefit ratio since the avoidance of H.I.V. outweighs any possible cardiac side effect

Red blood cells have 3 major functions: (1) transport oxygen from the lung to the tissue, (2) remove damaging oxygen radicals and (3) carry carbon dioxide CO2. from the tissue to the lung to be removed. The urgency of H.I.V. in donor blood necessitates the development of the simplest system in the shortest time. The most extensive clinical trials were based on Chang's glutraldehyde crosslinked polyhemoglobin (PolyHb) later developed by Biopure (Hemapure: bovine PolyHb) (24) and Northfield (human PolyHb) (21). They used the basic principle of glutaraldehyde crosslinked hemoglobin first reported by Chang (7) (Fig. 8). This has no blood groups and can be pasteurized to remove infective agents and can be stored at room temperature for more than 1 year. Large-scale clinical trials have been carried out including using human PolyHb in the ambulance without the need for typing or cross matching (21). Greenburg, Jahr and others have carried out clinical trials using (33) Hemapure:bovine PolyHb (23, 24). This has been approved for routine clinical use in South Africa to avoid the use of H.I.V contaminated donor blood (24). Other ongoing research includes the use of other sources of haemoglobin by Chen's groups with porcine Hb (21), Yang's group with Placental Hb (22), and Bulow's group and others with recombinant Hb (23).

(2) 2nd Generation: Oxygen carriers + removal of oxygen radicals:

Arterial obstruction can result in stroke and heart attack. Red blood cells, being 7 to 8 microns in diameter, have difficulty flowing through partially obstructed vessels to supply the needed oxygen. PolyHb, being a solution, can perfuse through to supply the needed oxygen. However, reperfusion with an oxygen carrier can release damaging oxygen radicals (Fig. 9).

D'Agnillo and Chang has prepared a soluble complex of Polyhemoglobin containing antioxidant enzymes to remove oxygen radicals (PolyHb-SOD-CAT) (25). It has the dual function of an oxygen carrier that can also remove oxygen radicals (Fig. 9). After 90 min of combined hemorrhagic shock and brain ischemia in rats, reinfusion of PolyHb-SOD-CAT did not cause brain edema (Fig.9) (26). On the other hand, PolyHb or a solution contain free Hb, SOD and CAT causes significant increases in brain edema. Ischemic small intestine releases damaging oxygen radicals when reperfused with PolyHb. However, PolyHb-SOD-CAT reperfusion does not increase oxygen radical release (Fig. 9). This is important during intestinal surgery or organ storage for transplantation.

The work of Hsia's group using conjugated hemoglobin containing synthetic antioxidants (PNPH) is another way to solve the problem (27). Another example is that of Alayash's group based on haptoglobin(28) Others included those of Simoni, Zal and other groups (23)

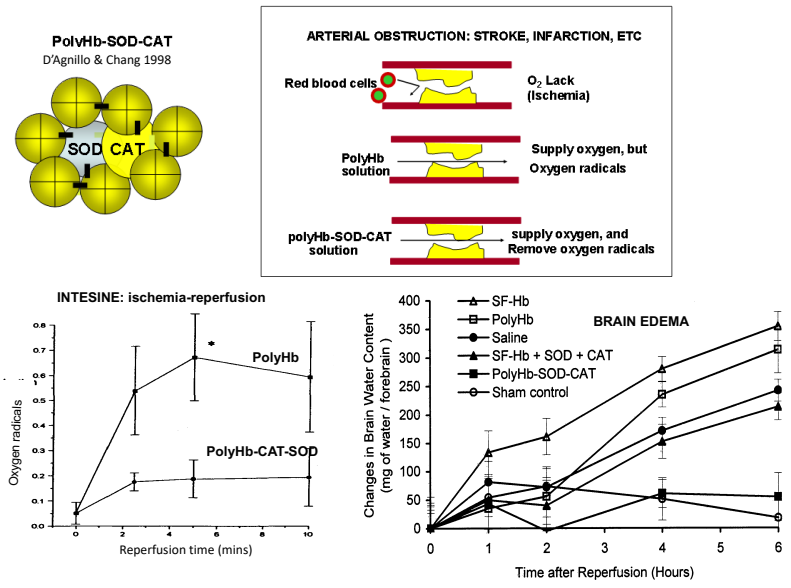
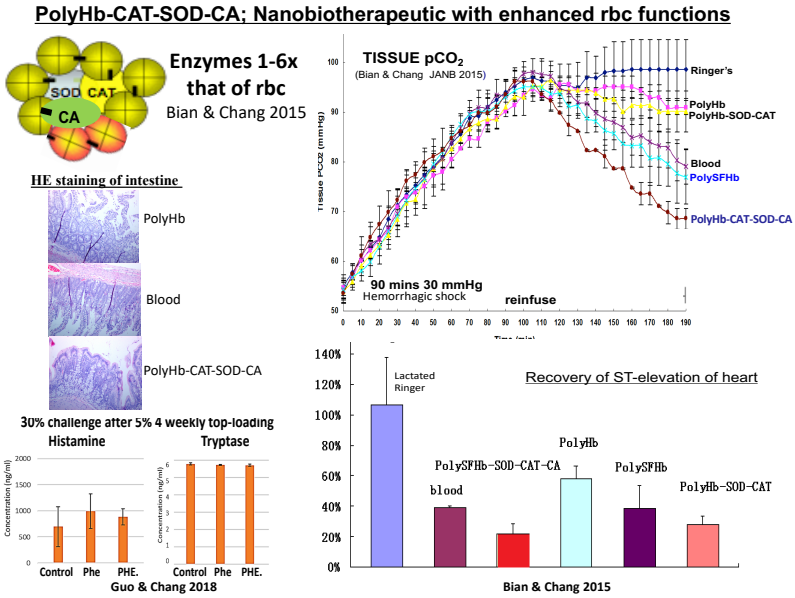
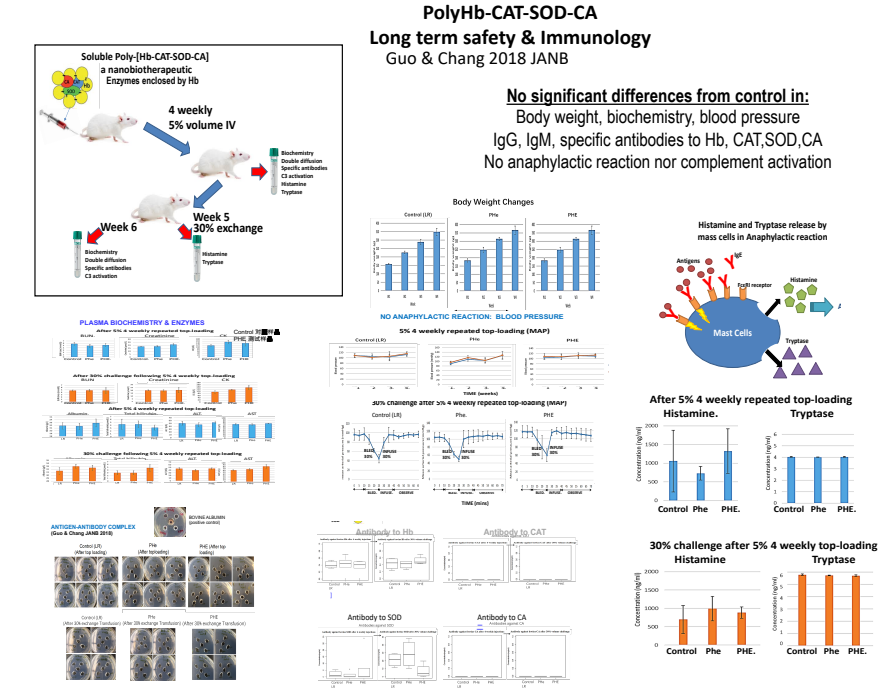


Fig. 9: ***Upper right:*** *Arterial obstruction can result in stroke and heart attack. Red blood cells cannot flow through. PolyHb, a solution, can perfuse through. (****Upper left****) PolyHb-SOD-CAT, a solution can perfuse through to supply oxygen and remove oxygen radicals* ***Lower right:*** *Unlike PolyHb, reinfusion of PolyHb-SOD-CAT does not cause brain edema in rat brain ischemia.* ***Lower left:*** *Unlike PolyHb, PolyHb-SOD-CAT reperfusion in ischemic small intestine does releases damaging oxygen*

(3) 3Rd Generation: All 3 rbc functions (Carries Oxygen + removes oxygen radicals + carries CO_2)

Other conditions as in sustained severe hemorrhagic shock may require all three rbc functions. We have designed a novel soluble nanobiotechnological complex (PolyHb-SOD-CAT-CA) (Fig). It not only has all 3 rbc functions, but it can have enhancement of all 3 rbc functions by increasing the concentrations of rbc enzymes in the complex (29). These rbc enzymes can be extracted from rbc inexpensively (30). This complex has no blood groups. The lyophilized preparation can be heat pasteurized at 68F for 2 h (31). This can be important if there is a need to inactivate H.I.V. virus, Ebola , COVID-19 virus, and other infective organisms. Unlike about 1 day for rbc at room temperature, this lyophilized preparation can be stored in room temperature for 320 days. Our result in a 90 minutes hemorrhagic shock animal model with 2/3 blood volume loss (Fig 10) (29) shows that it is superior to whole blood in the following ways: lowering of elevated intracellular pCO_2 , recovery of ST elevation, tropronin levels, lowering of elevated lactate, histology of the heart and intestine.





Long term study of bovine PolyHb-SOD-CAT-CA in rats shows safety and lack of immunological problems after 4 weekly 5% blood volume infusion followed by 30% volume exchange transfusion (32). This includes the measurement of histamine and tryptase that show no anaphylactic reaction (Fig. 10). Hemoglobin has very low antigenicity. Bovine PolyHb itself shows no immunological problems in patients (23,24). For PolyHb-SOD-CAT-CA the small fraction of enzymes are nanoencapsulated inside the large excess of hemoglobin molecules (36) (Fig.10)

(4) Nanodimension red blood cells

The original micro dimension artificial red blood cells are too large to survive in the circulation. Nanodimension artificial rbc is another way to solve this problem (33-35). Lipid membrane vesicles itself do not circulate well and the addition of PEG to the membrane to form a PEG-lipid-polymer membrane vesicle has increased the circulation time. At present, this approach at the late Tsuchida's group (35) is being continued by Sakai's group (34). In our laboratory we have been using an 80 nm mean diameter biodegradable PEG-Polylactide polymeric membrane nano rbc that contains all the rbc enzymes (Fig.11) (33). Both PEG-lipid and PEG-polylactide nano red blood cells can remain in the circulation longer than PolyHb or PolyHb-SOD-CAT-CA. However, they contain substantial amount of nonfunctional lipid or polymeric membrane. On the other hand, for soluble nanobiotherapeutic artificial rbc, PolyHb-SOD-CAT-CA, the "membrane" is functional in the form of oxygen carrying hemoglobin (Fig.11). Thus, each has its own advantage.

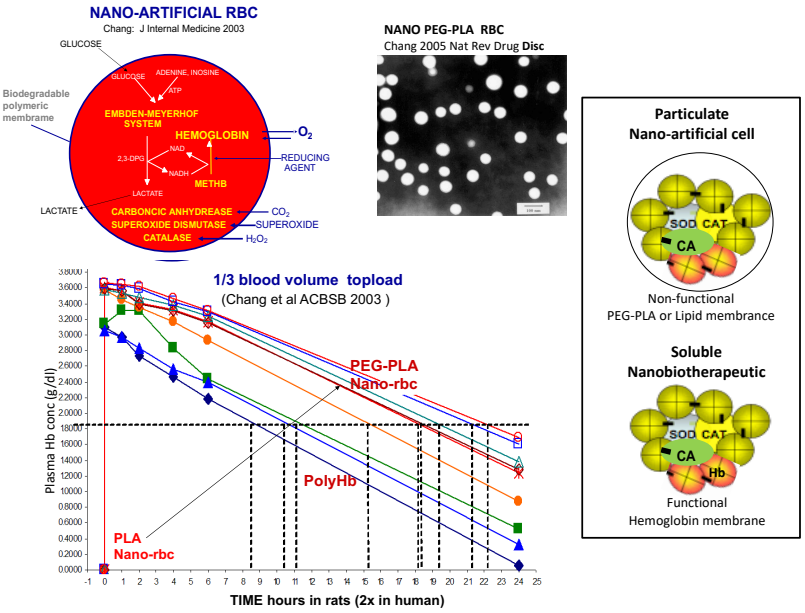
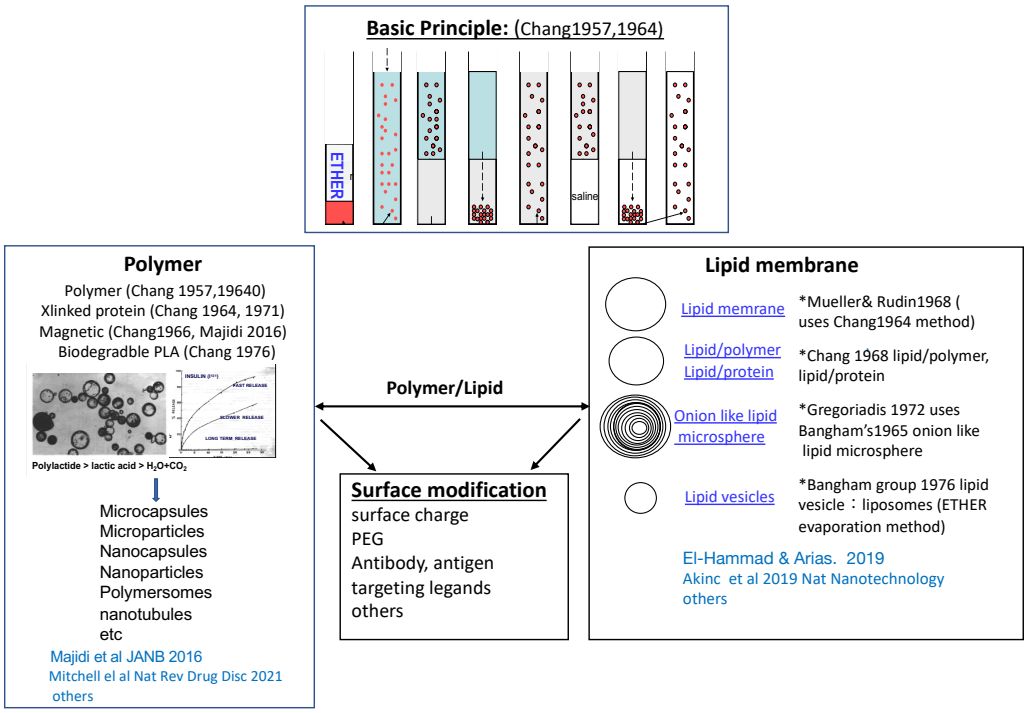


Figure 11: Upper left: *Nano artificial red blood cell (rbc) with biodegradable polymeric membrane and red blood cell enzymes.* ***Upper middle:*** *EM of PEG-PLA membrane nano artificial rbc with a mean diameter of 80 nanometer.* ***Lower left:*** *Circulation time of PEG-PLA membrane rbc in rats is 2x longer than Polyhemoglobin.* ***Right:*** *nano rbc contains substantial amount of nonfunctional lipid or polymer membrane. Soluble Hb nanoencapsulated nano rbc has functional oxygen carrying hemoglobin Updated from Chang (9, 10, 33) with copyright permission*

Future directions

International progress up to now shows that it is possible to tailor-make blood substitutes ranging from simple to complex (23). It is urgent to have these ready without again waiting until it is too late. We need to analyze the specific indications for 1,2,3 and 4 above. If a condition only needs oxygen, then there is no need to use a more complex one. On the other hand, it would be folly not to use a more complex one if indicated. We also need to intensify research on the many important ongoing research around the world. Examples include other novel approaches including novel crosslinkers; new sources of material from porcine, bovine, human cord rbc, recombinant, Arenicola marina; basic research on nitric oxide, oxidative stress, haptoglobin, rate of oxygen supply; safety and efficacy analysis and many other areas

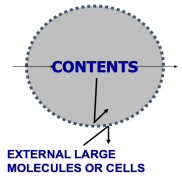
DRUG DELIVERY SYSTEM

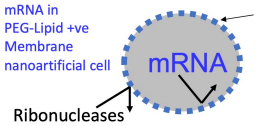


COVID_19 VACCINES

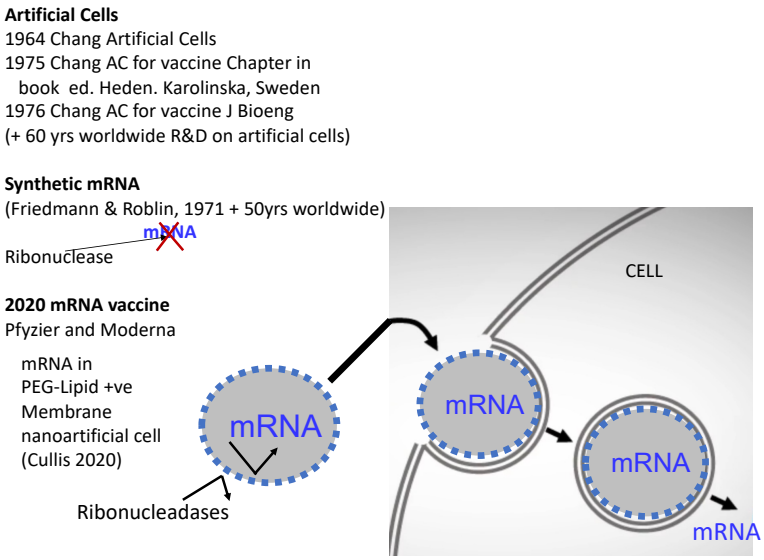
The first publication on the use of artificial cells for vaccine was in a chapter by Chang 1975 on Artificial Cells for vaccine (48) in a book edited by the late Prof Heden, Chair of Microbiology, Karolinska Institute, Sweden. It was too earlier for its time.

ARTIFICIAL CELL TECHNOLOGY.
Basic method:_Chang 1957-1965Intra
Micro, nano, contents, configuration,
Membrane of polymer and protein
Surface modifications
*Polysaccharide, +ve -ve charges, antigen (Chang 1964-e 1967)
*Polyethyleneglycol PEG
(Abuchowski & Davis 1970)
Lipid components
*Mueller& Rudin1968 (used Chang1964 method)
*Chang 1968 polymer-lipid
*Gregoriadis 1972 uses Bangham's
1965 lipid onion like membrane model
*Bangham group 1976 lipid vesicle：
liposomes (lipid evaporation method)
Biodegradable polymer
PLA (Chang 1976)
Combination of above technologies → 2020 mRNA vaccine Pfyzier and Moderna
Lipid-trace polymer, polymer trace lipid
Surface charge and PEG
PEG-Lipid, PEG-PLA,
PEG-PLA-lipid (Chang et al 2005 JANB)
PEG-+veLipid (Cullis et al 2021 Nature)
Others





ARTIFICIAL CELLS (AC) FOR THERAPY
1957 Chang (McGill) hemoglobin
1964 Chang (Science) Hemoglobin & enzymes
1968 Chang & Poznanski (Nature)
hereditary enzyme defects (mice)
1971 Chang (Nature) suppress lymphosarcoma in mice
1973 Chang et al (TASAIO & CMAJ) adsorbent for patients
1975 Chang for **vaccine** Chapter in book (Ed Heden)
1976 Chang (J Bioeng) for **vaccine**
1985 Chang (JANB), Palmer et al (Lancet) Lesch Nyhan patient
1986 Bourget & Chang (BBA) Phenoketonuria (rat model)
(1984 mRNA synthetised in lab)
1990 mRNA in lipid AC **vaccine** (mice)
1993 mRNA **vaccine** for influenza (mice)
(2005 mRNA modified to evade immune detection
But mRNA destroyed by Ribonucleases in Body)
2012. PEG-lipid AC mRNA **vaccine**(mice)
2016 PEG-lipid AC mRNA **vaccine** influenza (human)
2020 PEG-lipid positive surface charge AC mRNA
COVID-19 **vaccine** (Emergency authorization)
2020 AC adsorbent hemoperfusion for cytokine storm
in COVID-19 patients
2021 (Emergency Authorisation in Canada)



Fortunately, we and others around the world have been developing artificial cells as carriers for other biologics and biotherapeutics. This world have been developing artificial cells as carriers for other biologics and biotherapeutics. This allows Pfzier and Moderna to place synthetic mRNA inside nano-lipid artificial cells to prevent the enzymatic destruction of mRNA (Fig below)(48a.b,c). Other confiurations and formulations of artificial cells are being explored for other types of COVID_19 vaccines.

Bilayer lipid membrane artificial cells: liposomes

In 1965 Bangham reports the use of microspheres of onion-like concentric multilamellar lipid bilayers as membrane models in basic research (43). In 1968 Meuller and Rudin (44) reported that they use Chang's method (2) to prepare single bilayer membrane vesicles. A McGill PhD graduate, Gregoriadis, visits me before leaving for his postdoctoral fellowship in England. While there he becomes the first person to start the use of liposomes as drug delivery systems (45). However, onion like multi-lamellar liposomes limit the loading of water-soluble drugs. Thus, in 1976 Deamer and Bangham (46) report the use of an "ether evaporation" method to form single bilayer lipid membrane vesicles. This "ether evaporation method" is an extension of the 1957 Chang method using ether for the preparation of artificial cells (1,2) (Fig. 4). These lipid membrane artificial cells have since been extensively studied and used as drug delivery systems around the world (47). This is now a very successful approach for drug delivery. For the delivery of larger peptides, proteins and vaccines, the emphasis is using biodegradable polymeric system.

Biodegradable polymeric membrane artificial cells

Polylactide is biodegraded in the body to lactic acid and then water and carbon dioxide (Fig. 12) and is a F.D.A. approved material for medical implantation. Thus, in 1976 Chang reported the use of polylactide prepare biodegradable membrane artificial cells containing enzymes, hormones, vaccines and other biologics (37) (Fig. 12). Variations in the molecular weight of polylactides and thickness of the membrane and configurations can result in artificial cells that release insulin at different rate (Fig. 12). This approach has been extended and developed extensively world-wide as drug delivery systems in the form of nanoparticles, polymersomes or nanocapsules (37-41). Bowerman et al reported in 2016 that Docetaxel-loaded PLGA nanoparticles improve efficacy in taxane-resistant triple-negative breast cancer (40). Ravanshad, et al. in 2017 reported the use of nanoparticles in cancer detection by Raman scattering based techniques (41). Abed et al reported in 2018 the use of Lysozyme and DNAse I loaded poly (D, L lactide-co-caprolactone) nanocapsules as an oral delivery system (42)

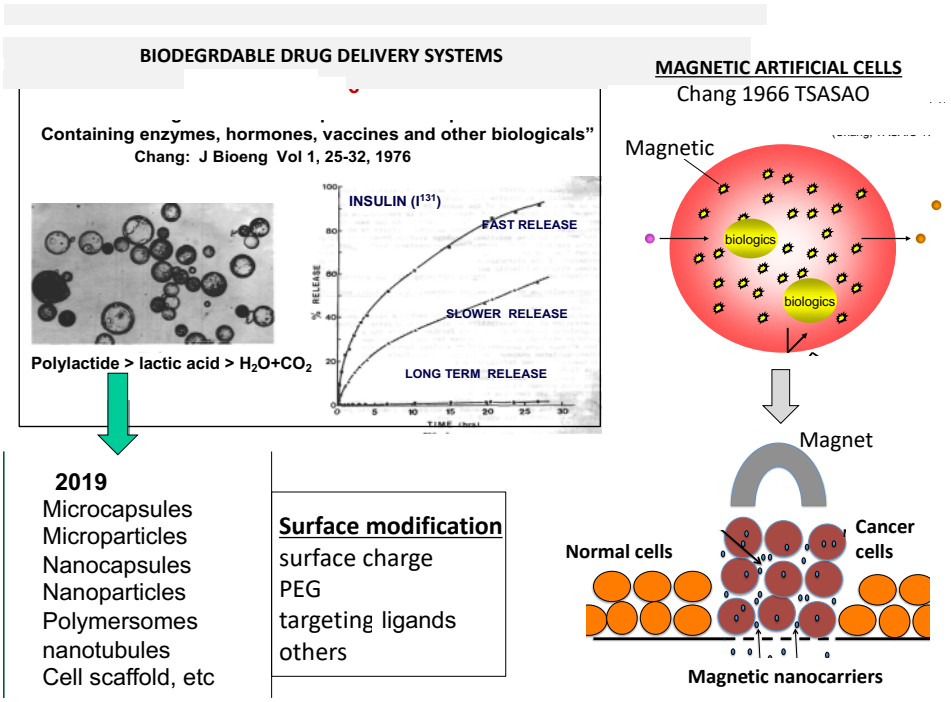


Fig. 12. ***Left:*** *Biodegradable membrane artificial cells containing enzymes, hormones, vaccines and other biologicals (Chang, 1976). Variations result in the release of insulin at different rates. Extended now to many different configurations and dimensions.* ***Right:*** *Artificial cells containing magnetic material.*
Updated from Chang (9, 10) with copyright permission

Targeting using surface ligands or magnetic properties and others

Back in the 1970, Chang's group has investigated the incorporation of surface charges, polysaccharides and protein onto the surface of polymeric artificial cells (Fig. 7) (2, 6). The most successful one is Davies of Enzon's use of Polyethylene glycol (PEG). PEG has been incorporated to both types of nano artificial cells to result in longer circulation time. Further developments lead to the incorporation of antibodies onto the polymeric or lipid membrane of artificial cells (Fig. 12), to allow for targeting to cells with the corresponding antigens. Brennick, C. A., et al. in 2017 report the use of neoepitopes as cancer immunotherapy targets (48). Artificial cells containing biological materials and magnetic materials have been prepared by Chang in 1966 (5) (Fig. 12). This way, external magnetic fields can direct their movement; remove or separate them from a mixture; retain them at specific site of action; stir or agitate them as in bioreactors, and other possibilities. This principle is now being used very extensively in bioreactors; in removing specific materials from a mixture as in diagnostics kits; in drug delivery systems; for locating radioactive material or chemotherapeutic agents at site of tumor and other areas of application. A 2016 review by Karkan et al on the use of magnetic nanoparticles for drug delivery is available. (49).

A more futuristic approach is Hu et al's 2018 report in Nature of Small-scale soft-bodied robot with multimodal locomotion with potential for drug delivery (50).

ENZYME AND GENE THERAPY

Enzymes inside artificial cells can act on external permeant substrates while avoiding protein sensitization, anaphylactic reaction, or antibody production with repeated injection (2-4, 6, 8, 9) (Fig. 13).

Chang's groups has been investigating the use of artificial cells for enzyme therapy since 1964 (2-10, 25,26, 29-33, 51, 54,55, 59, 60) (Fig.)

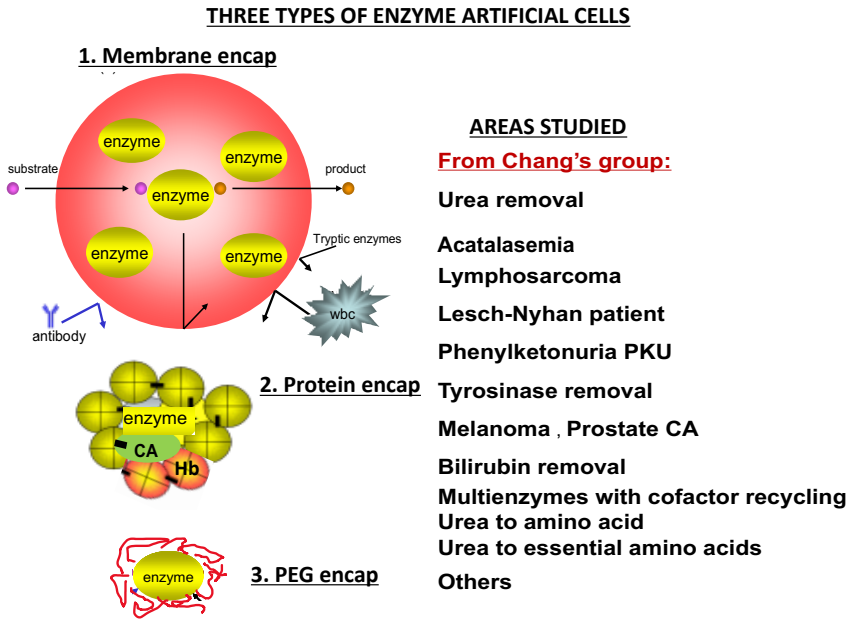
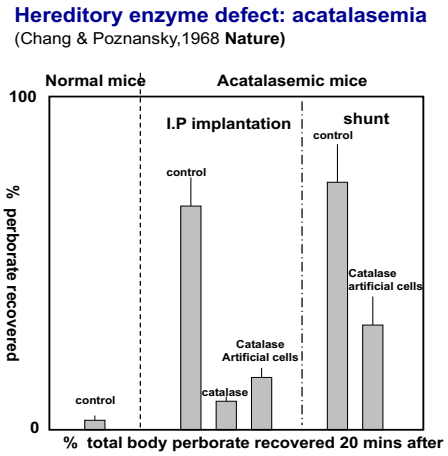


Fig. 13 **Left** *: Enzymes inside artificial cells, unlike those in free solution, do not have immunological problems. These can be in the form of membrane encapsulation, Neutral-protein encapsulation or PEG covering of the enzyme molecule.* ***Right:*** *This approaching has been studied for a number of medical applications. Updated from Chang (9, 10)*

Artificial Cells for Enzyme Therapy in Inborn Errors of Metabolism

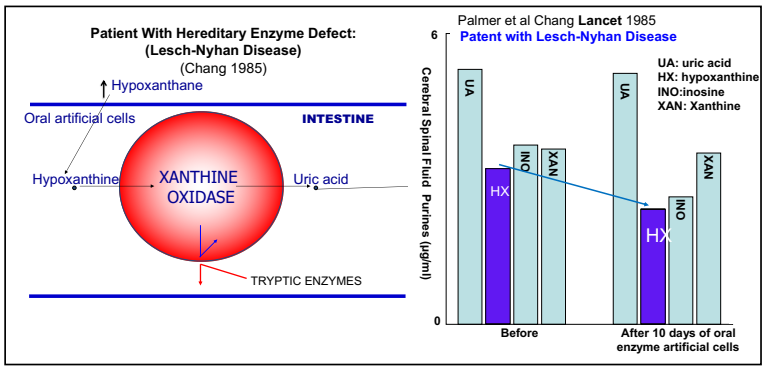
Acataemsemia



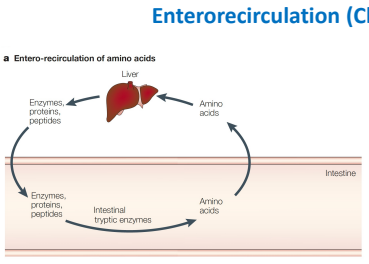
Implanted artificial cells containing catalase replaces the defective enzyme in mice with a congenital defect in catalase, acatalasemia (3). Unlike the free catalase, there is no immunological problem with repeated injections (51).

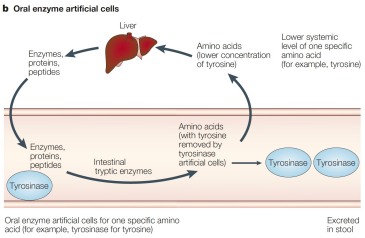
Artificial Cells containing Xanthine Oxidase for Lesch-Nyhan Disease

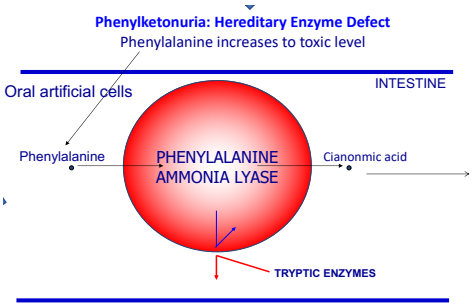
Lesch-Nyhan disease is an inborn of metabolism due to a deficient in a complex and unstable liver enzyme system. The Montreal Children Hospital contacted me that they have young child with severe symptoms due to Lesch-Nyhan Disease with elevated hypoxanthine and whether something could be done. The natural enzyme system in the liver is very complex and unstable. Fortunately, I found that there is a fermentation produced enzyme, xanthin oxidase and I was able to prepare artificial cells containing this simple stable fermentation produced enzyme, Xanthine Oxidase (Chang JABB 1985). We suspended this in jello or ice cream and the child enjoyed having this orally twice a day. After 10 days the system hypoxanthine was lowered (figure on left)(Palmer.. Chang, Lancet 1985)(55).



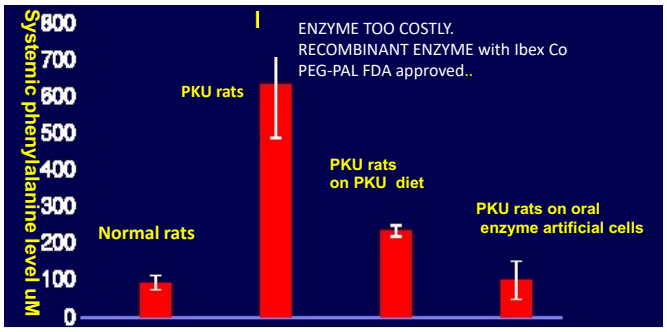
***Phenylketonuria PKU is the most common inborn errors of metabolism** due to hereditary enzyme defect of the very complicated and unstable multienzyme system in the liver.







We show that there is an extensive recirculation of amino acids in the intestine (Figure) (9.56) We therefore investigated the use of oral artificial cells containing a simple and stable single enzyme prepared by fermentation, phenylalanine ammonia lyase.(57). This is placed inside artificial cells and could effectively lowered the increased systemic phenylalanine to normal level in a PKU rat model. Thus, we show that orally administered artificial cells containing phenylalanine ammonia lyase (PAL) lower the systemic phenylalanine levels in phenylketonuria (PKU) rats and improved the growth of the animals (57). We then seek a company to develop this for clinical use. They in turn collaborate with another company and develop **an injectable PEG-phenylalanine ammonia lyase that has just been approved** by **FDA for use in adult PKU patients** (58,59). In order to avoid long term injection, further development should be carried out for a formulation for oral administration (57) as we have done in the laboratory.



In the same way, our study shows that oral artificial cells containing tyrosinase when given orally lowers the systemic tyrosine level (9). Kaminsky

et al use argocytes containing enzyme nanoparticles to reduce toxic concentrations of arginine in the blood (63). Abed et al reported in 2018 the use of Lysozyme and DNAse I loaded poly (D, L lactide-co-caprolactone) nanocapsules as an oral delivery system (42)

ARTIFICIAL CELLS FOR CANCER THERAPY

CANCER IMMUNOTHERAPY

This has become an explosive area. 。

But There are many problems that need to be solved.

Need for suitable delivery systems

Riley, R.S., June, C.H., Langer, R. *et al.* Delivery technologies for cancer immunotherapy. *Nat Rev Drug Discov* **18,** 175–196 (2019).
Martin, J.D., Cabral, H., Stylianopoulos, T. *et al.* Improving cancer immunotherapy using nanomedicines: progress, opportunities and challenges. *Nat Rev Clin Oncol* **17,** 251–266 (2020)

Need a less severe method especially for the less severe cases

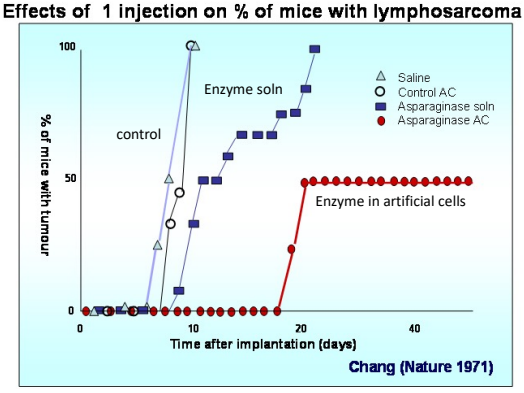
Wang, J., Dong, R., Wu, H. *et al.* A Review on Artificial Micro/Nanomotors for Cancer-Targeted Delivery, Diagnosis, and Therapy. *Nano-Micro Lett.* **12,** 11 (2020)

Enzyme therapy using nanobiotherapeutic inside polymeric-lipid nanoartificial cells

Wang & Chang JANB 2021

Lymphosarcoma in mice

Asparaginase artificial cells for lymphosarcoma.
Chang 1971 Nature
Effects of 1 injection on % of mice with lymphosarcoma

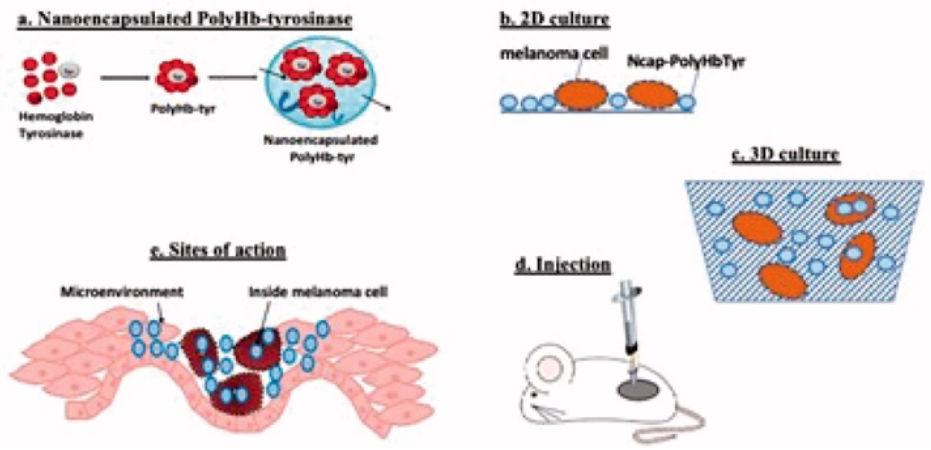


Do not require long term use
Now in clinical use by other groups as
PEG-asparaginase for acute leukemia (Wetzler et al 2007)

Implanted artificial cells containing asparaginase delay the onset and growth of lymphosarcoma in mice (4). This has been extended by other groups using PEG-asparaginase for the treatment of leukemia in patients (54).

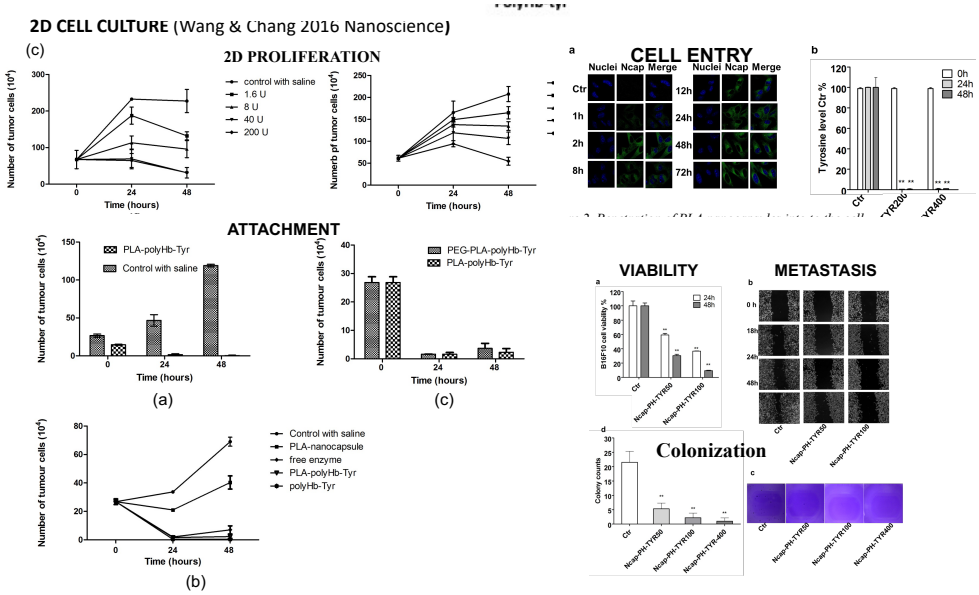
Enzyme therapy for Melanoma Based on nanobiotherapeutic artificial cells

Melanoma is a deadly skin cancer. Surgery is effective for early stages but there may be remnant cells. Treatments of later stages with immunotherapy and chemotherapy are very promising but are associated with severe side effects. Moreover, a dangerous type of melanoma cannot be detected early enough for surgery. There is an urgent need for treatment with less severe side effects.



Polyhemoglobin-tyrosinase effectively lowers systemic tyrosine and delayed the growth of melanoma in mice (60). In order to suppress the growth of melanoma, we use a novel system of artificial cell polymer–lipid membrane nanocarrier containing a biomolecular nano-system of enzyme–oxygen biotherapeutic (61).

We started first testing this in a 2D culture system (Fig top left) that shows its effectiveness (61)

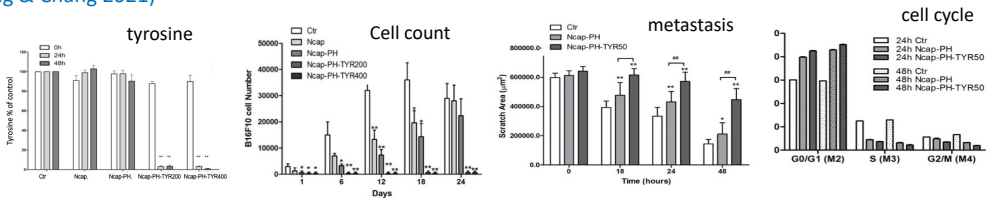


We then show (1) its effectiveness and mechanisms in inhibiting the growth of melanoma in a 3D culture collagen medium that is more similar to that in the animal. (2) This allows us to design and

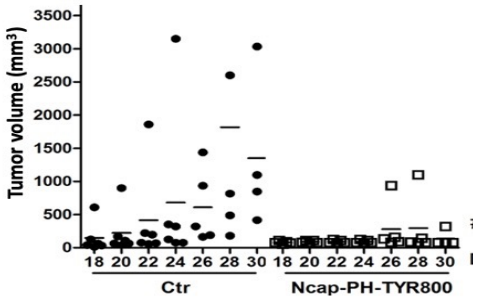
carry out animal studies to successfully show that this can inhibit the growth of melanoma in an animal model. This includes following the tumour sizes and body weights every 2 days for 30 days followed by histology of the sites of injection and vital organs. We also analyze the action of the different components of the nanocarrier–nano-biotherapeutic complex. In conclusion, the results show the safety and clinical feasibility of this approach in the animal model (62)

Nanobiotherapeutic
In PLA-nanoartificial cells
(Wang & Chang 2021)

3D culture (Wang & Chang 2021)



Effect on the growth of B16F10 melanoma tumor in mice
Wang & Chang (2021 JANB)



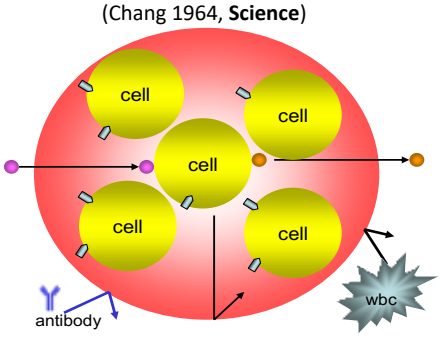
	Nodule tissue	Viable tumor % (estimate)	Necrosis % (estimate)	Hemorrhage % (estimate)	Nanoparticles visible	Granuloma reaction	Necrotic blood vessels
Ctr	Neoplastic	65	30	5	-	-	+/-
Ncap-PH-TYR800	Granuloma	0	0	0	+	+/-	-

Artificial Cells Containing Biological Cells

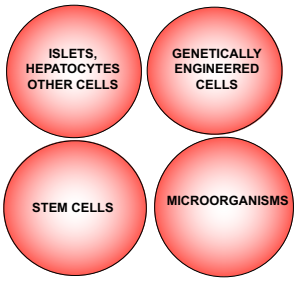
Present status

The first artificial cells containing intact biological cells were first reported by Chang in 1964 (2) using the drop method. It was proposed that "protected from immunological process, encapsulated endocrine cells might survive and maintain an effective supply of hormone" (Fig) (6).

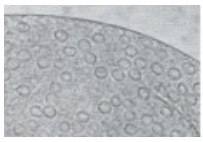
Cell encapsulation
(Chang 1964, **Science**)



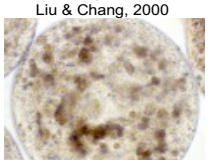
ARTIFICIAL CELL CONTAINING CELLS
Extensive efforts around the world



Cells in AC
(Chang 1965, Chang et al 1966)



Stem cells and Hepatocytes in AC
Liu & Chang, 2000



Cell scaffold
(Langer et al 2000)

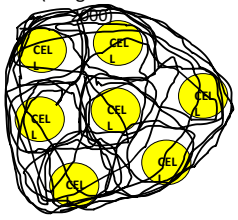
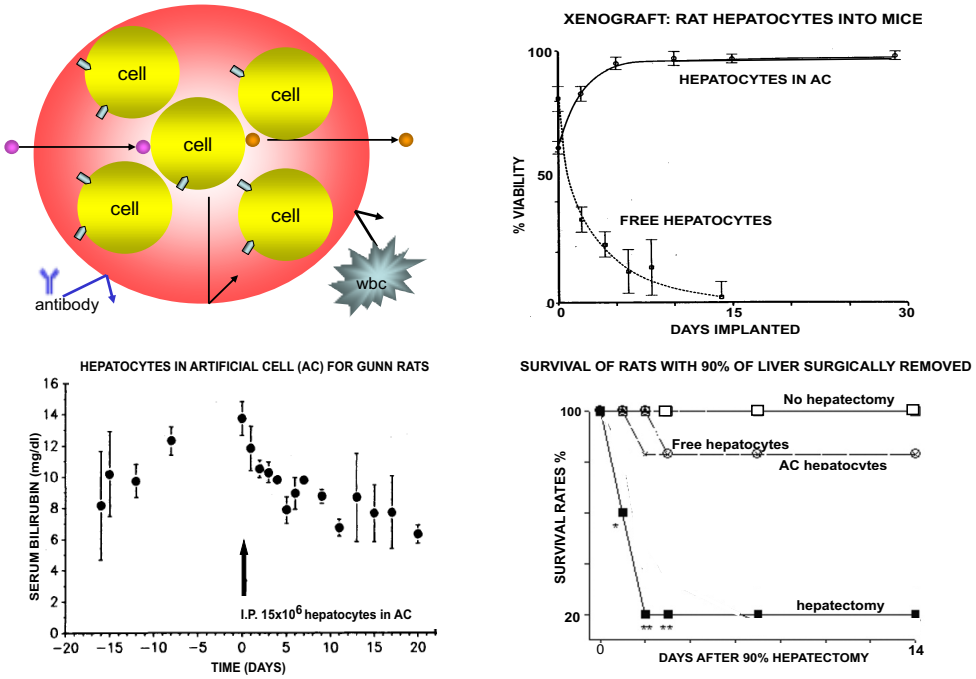


Fig.. Upper left: *Cells inside artificial cells protected from outside.*
*. **Lower**: Cells can be bioencapsulated inside artificial cell or entrapped in scaffold of fibers or nanofibers **Upper right**: Bioencapsulation of islets, cells, genetically-engineered cells.*

I he*lp* Conaught Laboratory to enclose islet in artificial cells for use in diabetes (64). This basic principle has been extensively developed around the world for cell therapy (8,9, 64-77). Examples include artificial cells containing endocrine tissues for instance, islets for diabetes. Another extensively investigated area is artificial cells containing genetically engineered cells for a number of clinical conditions. His own laboratory has investigated artificial cells containing liver cells (figure below)(9, 68). result in animals have been promising. However, one implantation can only function for less than one year, and this is not practical for long-term illness like diabetes. Repeated injections would have retention problems.

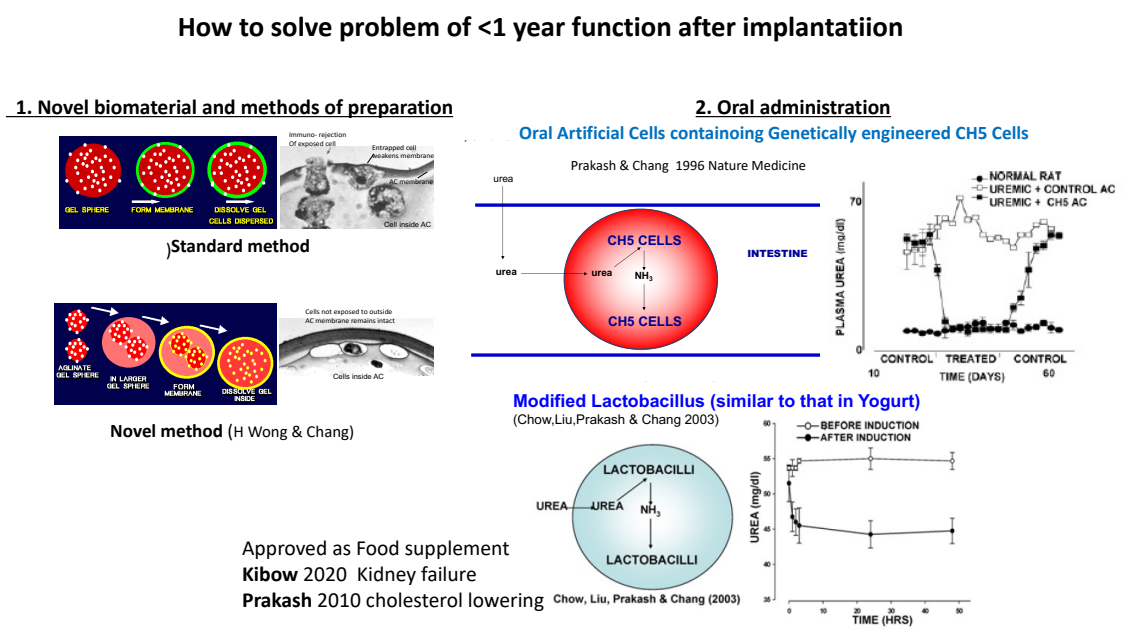
Artificial cells containing Hepatocytes
(Chang, Wong & Chang, Bruni & Chang, Liu & Chang from Chang 2007)



There are at present 4 ways to solve this problem :

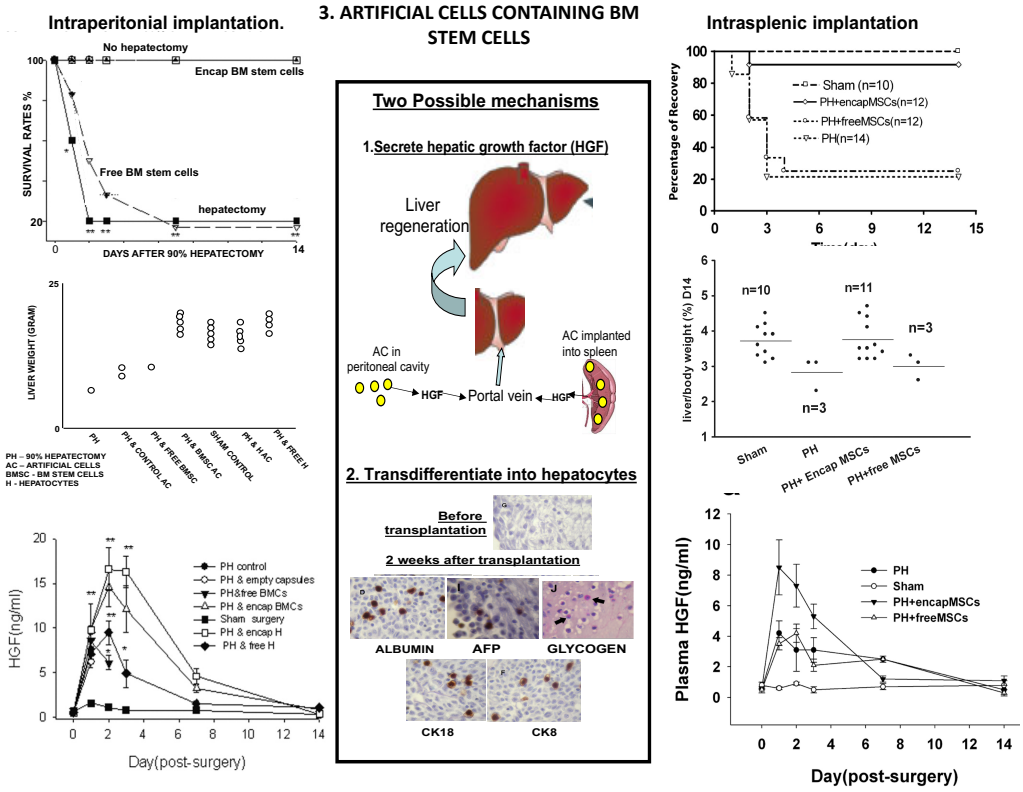
(1) Improved **biomaterials** with better long-term biocompatibility and improvement in the **method of preparation** as shown in Figure below

(2) **Oral administration of artificial cells containing microorganisms** Garofalo & Chang in 1991 show the effectiveness of artificial cells containing microorganisms for the in vitro removal of serum cholesterol (69). In 1996, Prakash and Chang (70) show that artificial cells containing genetically engineered E. coli DH5 cells given orally to kidney failure rats effectively lower the elevated blood urea



level. Even though Reardon in his 2018 Nature paper (71) supports the use of genetically modified bacteria in the fight against diseases, regulatory agencies are still hesitant about the use of genetic engineered microbes. In anticipation of this **Chang asks his group in 2003 (72) to use artificial cells containing modified lactobacilli, since lactobacilli are being safely used in Yogurt. This also avoids the use of genetically engineered microbe and allows the safer use for oral administration in human**. Prakash's group has since carried out extensive research into the use of this approach for clinical use in patients (73)

(3) **Use in regenerative medicine** that only need months of function, for example the use of artificial cells containing bone marrow stem cells in liver regeneration. Liu and Chang (74,75) study this in rats using artificial cells containing bone marrow stem cells. When implanted into 90% hepatectomized rats, this increases the recovery of the rats to 100% vs 11% in the control group and 33% in the free bone marrow stem cells (Fig.). Artificial cells containing stem cell can also be implanted into the spleen to carry this function (76)



(4) The use of biodegradable **scaffolds** started by Langer , Sefton and other groups, this is now a very popular and exciting approach. Grant's 2018 review (78) shows that this

is now a very promising and active area. Biodegradable scaffolds are prepared in the shape of specific tissue or organs. The cells are seeded into the scaffold and allow to grow in the scaffold until they reach the required shape and dimension and take over the biodegraded scaffold support.

TOWARDS MORE COMPLEX ARTIFICIAL CELLS

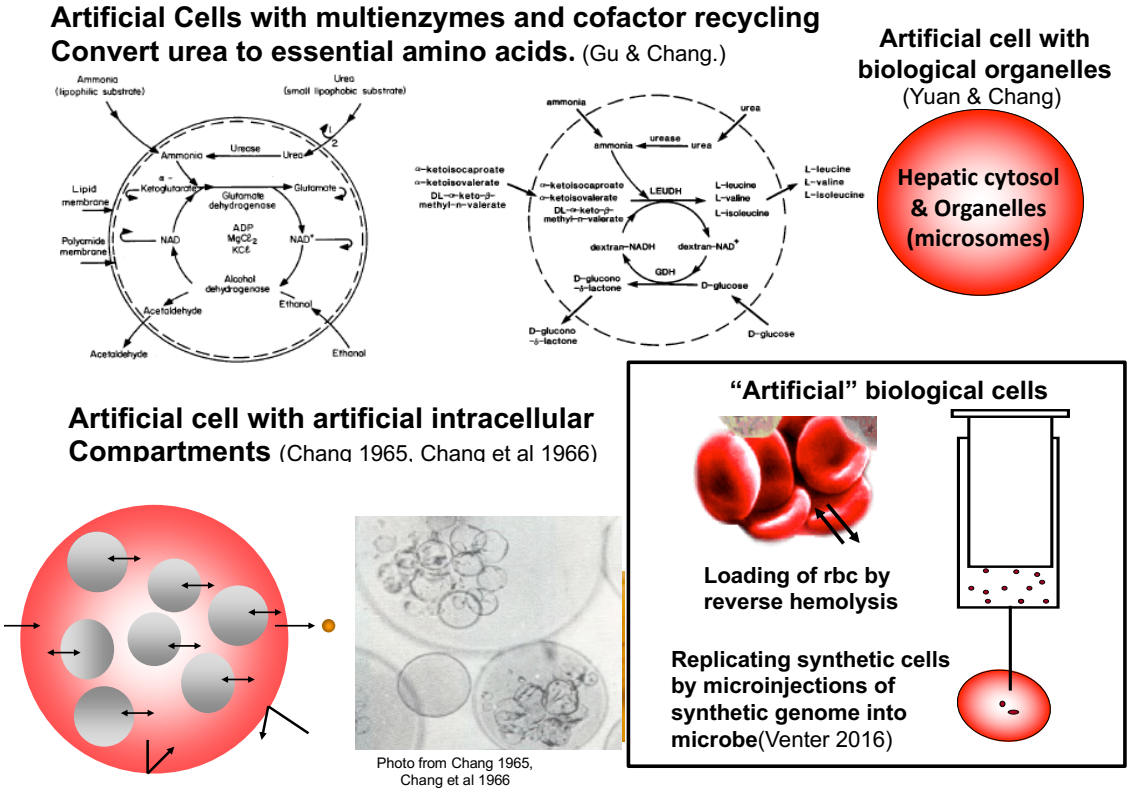


*Fig. . **Upper :**. Artificial cells containing multienzyme systems with cofactor recycling can convert waste, urea, into useful essential amino acids, **Upper right:** Artificial cells that contain liver cytosol and organelles like microsomes) **Lower right:** Reverse hemolysis to load red blood cells with drugs. Microinjection to introduce synthetic DNA into microbes.*

Multienzyme systems with cofactor recycling

Most enzymes in the body function as multienzyme systems with cofactor recycling. Gu and Chang (79) have prepared artificial cells containing multienzyme system with cofactor recycling and show that they can be used to convert metabolic waste like urea and ammonia into essential amino acids (Fig.). The cofactor, NADH, can be retained inside the artificial cells in the form of NADH-dextran or by the use of lipid–polymer membrane. We have also included all the multienzyme system of red blood cells inside nanodimension artificial red blood cells (33).

Artificial Cells with Intracellular Compartments

Biological cells contain organelles that allow for more effective compartmental function. We have prepared artificial cells with intracellular compartments (6, 8,9,80) (Fig.). This can allow for more efficient stepwise enzymatic or other biological functions. This principle has been extended for possible use in therapy by Hosta-Rigau and Stadler (81).

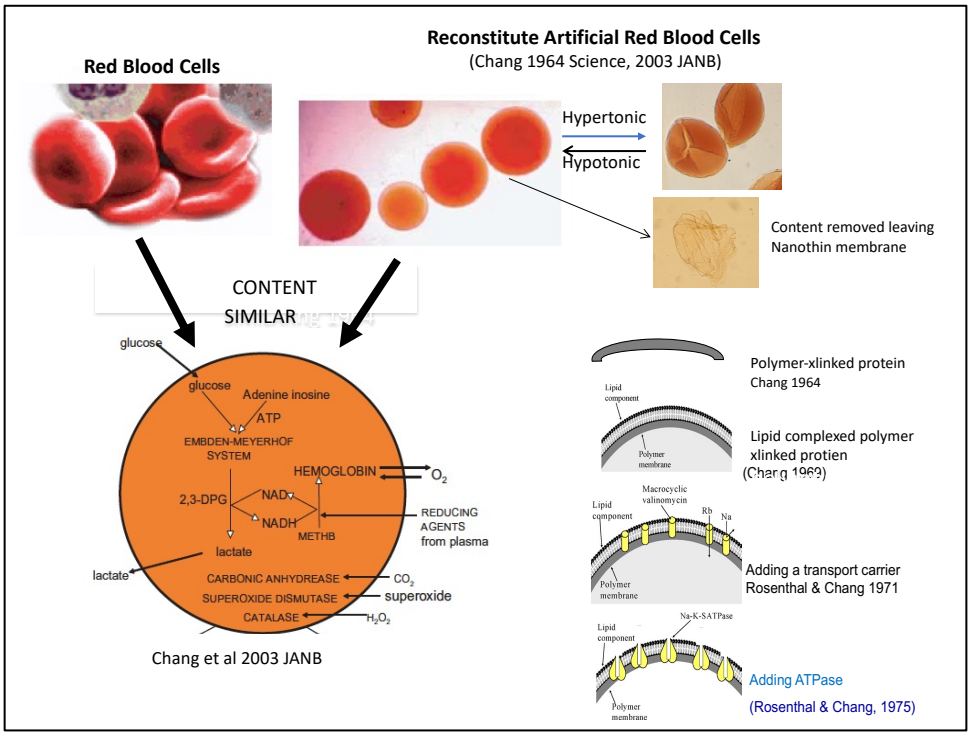
Artificial cells containing microsomes, cytosol, ribosome and polymerase

Yuan and Chang isolate microsomes and cytosol from rat liver and encapsulated into polymeric membrane artificial cells (9, 82,). 20NADPH-cytochrome C reductase and lactate dehydrogenate are used as the marker enzymes for respectively microsomes and cytosol and show retention of activities. Monnard and Deamer (83) prepare models for primitive cellular life by encapsulating T7 RNA polymerases and templates into lipid membrane artificial cells, lipid vesicles. They can synthesize an RNA transcript from the DNA template. This is a slow process because the lipid membrane has low permeability to the needed 4 nucleoside triphosphates. Oberholzer et al encapsulate a complex polymerase system into liposomes and show that the PCR reaction could be carried out (84). The problem is again the low permeability of the lipid membrane to the needed substrates. They have also encapsulated ribosomes into liposomes and obtain some translation product. More permeable polymeric or lipid-polymer membranes may shove these permeability problems. In another study, Griffiths and Tawfik (85) use compartmentalization to load the transcription/translation system in a water-in-oil emulsion. This way each gene can occupy a separate water emulsion to carry out its function. Artificial cells containing "subcellular compartments" can be another possible way of doing this (6) Fig)

Synthetic genome for replicating synthetic cells

After extensive research, in 2016 Venter's group report in Science their successful preparation of a synthetic minimal bacterial genome (86). Instead of synthetic membrane, by microinjection they have ingeniously make use of the complete membrane of the microbe. By doing this, they are able to prepare replicating cells using their synthetic genome.

Reconstruction of complete biological cells



A 2018 special issue in Nature concentrates on the feasibility of constructing complete biological cells (86.87).Red blood cells are the simplest of all human cells. It is one of the most important group of cells, since without them, we cannot survive. As described above, we have already prepared complete artificial red blood cells. (Fig on left) Researchers are now interested in doing this for the more complicated types of cells as discussed by Gopfrich et al in 2018 (88)

NONMEDICAL USES OF ARTIFICIAL CELLS

There are many developments and uses of the principle of artificial cells for agriculture, bioreactors, cosmetics, food production and aquatic culture (89).

Another area is the use of artificial cells in nanorobatics and nanocomputers that in 2004 becomes the European Commission sponsored Programmable Artificial Cell Evolution (PACE) and in 2008 becomes the European Centre for Living Technology (90).

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FUTURE OF ARTIFICIAL CELLS

The following prediction in Chang's 1972 monograph on "Artificial Cells" (6) is already out of date: *"Artificial Cell is not a specific physical entity. It is an idea involving the preparation of artificial structures of cellular dimensions for possible replacement or supplement of deficient cell functions. It is clear that different approaches can be used to demonstrate this idea*". Artificial cells have now already progressed way beyond this 1972 prediction. Artificial Cell has already led to development and approval for routine clinical uses in a number of areas:

- For use in COVID_19 vaccines.
- Hemoperfusion for COVID-19 cytokine storm treating poisoning, partial support of liver and renal failure, and for some immunological diseases.
- For use as first-generation blood substitute in countries with HIV contaminated donor blood.
- As a number of drug delivery systems.
- PEG-asparaginase for use in leukemia treatment.
- Recently approved as PEG-Phenylalanine ammonia lyase for the treatment of adult PKU.

Even then, we have only just touched the surface of the potential of artificial cells. One hopes that the many arbitrary subdivisions of "artificial cells" under the guise of different names can come together! When this takes place, the result of the pooling of talents, specialized know-how in this very interdisciplinary and international area will lead to progress beyond anyone's imagination [8,9,12,91].

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