

CHAPTER 1

50th Anniversary of Artificial Cells

This first chapter is not a scientific chapter. It is a personal “story” on the occasion of the 50th Anniversary of the first report on artificial cells, aimed at answering the following questions. Why artificial cells and what are they? Where and how were they first prepared? What is the time line of the new ideas related to artificial cells since they were first reported 50 years ago?

1.1. Background

Back in the 1950s, Professor F.C. MacIntosh, chairman of Physiology, and Sir Arnold Burgen started a special “honors physiology” program in the faculty of medicine at McGill University. The farsighted and challenging program consisted of advance cell physiology combined with advance courses in polymer chemistry, physical chemistry and radiation chemistry. I was one of the four chosen to be the “guinea pigs” for this new program and it started my interest in applying basic research to medical treatment. The problem was where to start.

We all feel humble in the face of the ingenuity and complexity of nature. Yet, it need not prevent us from studying, examining and even attempting to prepare clinically useful systems having a few of the simpler properties of their natural counterparts. Indeed, working on a molecular level, researchers at that time had already synthesized a number of biological molecules.

However, on the cellular level, despite the basic importance of cells, no one seemed to be interested in “artificial cells.” Perhaps this was premature, since our basic knowledge of biological cells was still

incomplete. Yet, organ substitutes like Kolff's artificial kidneys were hardly an exact replicas of their biological counterparts. Despite this, these substitutes were already an accepted method of treatment for kidney failure patients at that time. Thus, I thought that to prepare clinically useful artificial cells, one might not have to prepare replicas of biological cells.

1.2. Starting with Artificial Red Blood Cells

Being an uninitiated beginner, I thought that one can easily prepare artificial red blood cells for use in patients. After all, red blood cells are one of the simplest biological cells. Furthermore, there are practical reasons for doing this. Red blood cells are the best material for use in transfusion, but as shown in Fig. 1.1, there are a number of problems involved. Artificial red blood cells would solve many of these problems (Fig. 1.1).

When I asked around for a method to do this, I was politely told that I was asking for the impossible task of preparing a water emulsion suspended in water. Most people were also taken back by this "far-fetched" idea. Fortunately, my parents have instilled in me the sense that hard work and determination can turn impossible ideas into possibilities. They cited my grandfather as an example of someone who was able to build a manufacturing empire in Swatow (Shantou), starting with nothing as a poor preacher's son. Thus, I quietly and stubbornly started some simple experiments in my dormitory room at McGill's Douglas Hall of residence. Beginner's luck plus the excellent teaching in advance cell physiology and advance chemistry had allowed me to prepare some very crude artificial red blood cells.

I showed this preliminary result to Sir Arnold Burgen and he was most enthusiastic. He helped me persuade the chairman, Professor MacIntosh, to let me use this research for the required honors physiology research project. The department assigned me a corner of the teaching laboratory. By working out a drop method to first prepare larger artificial red blood cells (Fig. 1.2), I was able to use this principle, but using emulsification to prepare microscopic artificial red blood

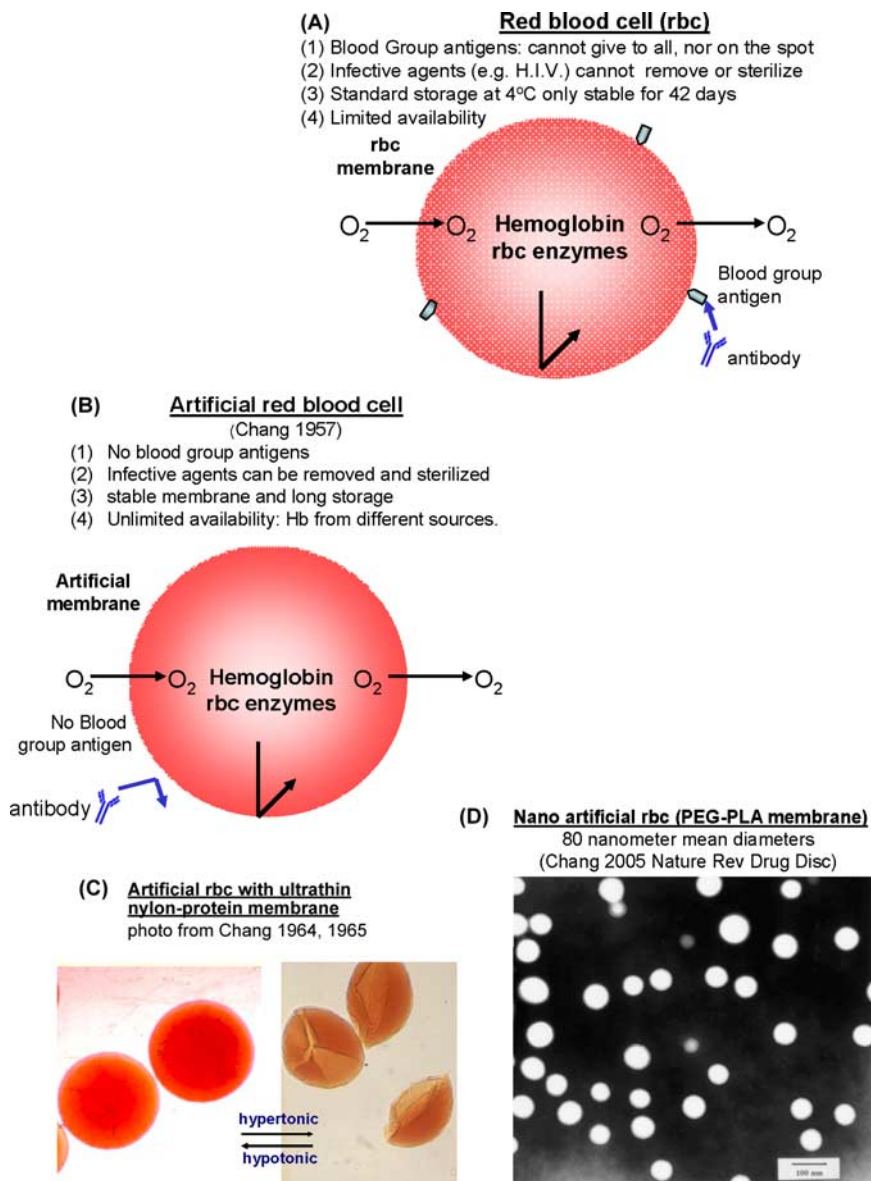


Fig. 1.1. (A) Characteristics of red blood cell. (B) Characteristics of artificial red blood cell. (C) Artificial rbc with ultrathin nylon-protein membrane. Spherical in hypotonic solution, becoming “crenated” in hypertonic solutions. Reversible when moved from one solution to another. (D) E/M of nanodimension (80 nanometer) artificial red blood cells containing Hb & rbc enzymes.

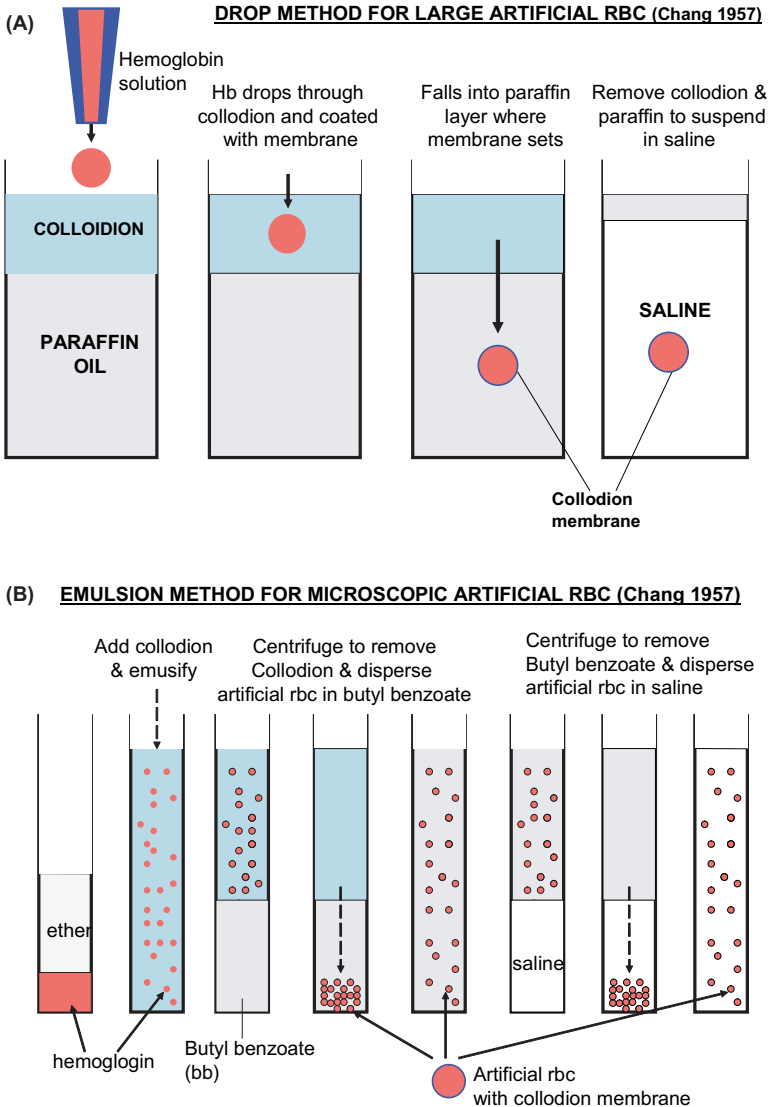


Fig. 1.2. Original (1957) method of preparing artificial cells (see Appendix I for details). (A) Drop method for preparing large artificial cells. Principle later extended for use in bioencapsulation of cells, stem cells, genetic engineered cells. (B) Emulsion phase separation method for preparing microscopic artificial cells (unlike the above, “collodion” prepared by removing most of alcohol and replaced with ether — see Appendix I). Principle extended to method for preparation of microscopic artificial cells and drug delivery systems and nanodimension artificial cells (Chang, *Nature Rev Drug Discovery*).

ARTIFICIAL CELLS

- Biotechnology, Nanomedicine, Regenerative Medicine, Blood Substitutes, Bioencapsulation, and Cell/Stem Cell Therapy
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<http://www.worldscibooks.com/lifesci/6395.html>

cells (Fig. 1.2). For the grading of this research project, the department asked for a sample of the artificial red blood cells. It was a relief when Professor Sekelj's analysis supported my oxygen dissociation curve and the research report was approved (Chang, 1957).

The extrusion drop method has become the basis for extension and modifications of methods for preparing artificial cells to encapsulate biological cells. The emulsion method has also become the basis for further extensions and modifications of the methods for the preparation of artificial cells of microscopic or nano dimension in nanotechnology. Because of this, the complete report including methods of preparation is reprinted in Appendix I in this monograph for interest and reference. The original project report has always been available in the McGill Medical Library. Part of this report was published in the 30th Anniversary Symposium volume (Chang 1988c).

1.3. Further Research and the First Routine Clinical Use of Artificial Cells

Over the next 5 years of my medical school and internship, the department continued to let me use the teaching laboratory space, providing me with summer salary support from the Faculty of Medicine. After this, since no one at McGill was carrying out related research, a Ph.D. committee was put together. In addition, Professor MacIntosh wrote a supporting letter for my submission to *Science* as the sole author of this work. This was under the condition that I use the title "semipermeable microcapsules" instead of my original pretentious title of "Artificial cells." *Science* accepted and published the paper (Chang, 1964). The Ph.D. thesis was also entitled "Semipermeable aqueous microcapsules" instead of "Artificial cells" (Chang, 1965). It is interesting to note that no one, including myself, knew precisely what "semipermeable microcapsules" meant. The outcome was that very few researchers followed up on this research for many years to come! The delay of others entering this line of research means that most of the earlier research in this area has been carried out in my laboratory.

In addition to the operating grant and career investigator award from the Medical Research Council of Canada (the present Canadian Institutes of Health Research), I was later awarded a large special program grant by the council. I took this as a challenge to show that all my findings about the potential implications of artificial cells can actually be put into clinical practice. With this, I was able to develop artificial cells which contain absorbents for use in hemoperfusion for the treatment of suicidal patients or patients involved in accidental use of sleeping pills and other medications. However, this being a very new approach, other physicians were initially concerned about medical liability. Thus, first worked out a way to scale up the preparation. After this, I carried out preclinical animal safety and efficacy studies as well as clinical treatment of patients. This required a lot of work and was highly time consuming; it was possible because of the unselfish support of my wife, Lancy, who knew the importance of this work for patients. Among many other things, she waited for more than 10 years before our first honeymoon. The result was hemoperfusion based on artificial cells, being carried out routinely around the world for the treatment of suicidal and accidental uses of medications in adult and pediatric patients.

In the meantime, I was completing an invited monograph for Charles C. Thomas Publisher and it contained a detailed description of methods. On becoming a tenured full professor in 1972, I immediately had this published (Chang 1972a). The advantage of having “tenure” is that it enabled me to safely use the title “Artificial cells” instead of “Semipermeable microcapsules” for this monograph! This self explanatory title and the detailed description of methods, combined with interests in the clinical use of artificial cells in hemoperfusion, led to a sudden surge of research activities in this area around the world. However, at that time the industries were mainly concerned with the development and use of artificial cells for hemoperfusion and drug delivery systems.

1.4. Importance of Progress in Parallel Areas of Biotechnology, Molecular Biology, and Regenerative Medicine

Most of my original ideas and basic research were related to enzyme and gene therapy, cell therapy, blood substitutes, regenerative medicine, nanomedicine and related areas. Developing these for actual clinical use required parallel developments in molecular biology and biotechnology that were not yet available. More recently, many groups around the world have made exciting progress in biotechnology, molecular biology, genetic engineering and related areas. The outcome is a recent new wave of research and development in artificial cells. Many groups around the world are now working on extensions and modifications of artificial cells for use in nanotechnology, nanobiotechnology, blood substitutes, regenerative medicine, gene therapy, cell/stem cell therapy and other areas (Orive *et al.*, 2003; Chang, 2005). Later chapters will describe some of these in more detail. This is now such a broad area that space will only allow for review of these areas with detailed examples based on research in my laboratory. Research in the many other centers will be presented by other authors in subsequent publications under the book series on "Regenerative Medicine, Artificial Cells and Nanomedicine."

1.5. Historical Milestones

Table I shows examples of the milestones of the first report of original ideas in artificial cells. This is not a complete listing and more details will be given in subsequent chapters.

Table 1.1 Artificial Cells (AC): Time Line of Ideas Since First Reported (1957)

1957 Chang	First artificial cells prepared with a synthetic membrane to replace RBC membrane and containing hemoglobin and red blood cell enzymes (emulsion phase separation, extrusion method or spray coating)
1964 Chang (<i>Science</i>)	Artificial cells (AC) containing enzymes, hemoglobin and cells formed by interfacial coacervation or interfacial polymerization to form membranes of polymer, crosslinked protein, polymer conjugated with protein, also crosslinked protein microspheres
1964, 1965 Chang	Nanobiotechnology: crosslinked protein (PolyHb) & conjugated Hb
1964, 1965 Chang 1966: Chang <i>et al.</i>	Extrusion drop method for AC to encapsulate intact cells for immunoisolation in cell therapy
1965 Bangham <i>et al.</i>	Liquid crystal microspheres of multi-lamellar lipid (liposomes) as membrane model for basic research
1965, 1972a, 1973b Chang	AC for molecular sieve chromatography and separation
1965 Chang 1966 Chang <i>et al.</i>	AC with intracellular multi-compartments
1966 Chang	Silastic AC and microspheres containing protein
1966 Chang	AC containing magnetic materials and biological materials
1966, 1969a Chang	Ultrathin membrane AC containing adsorbents for hemoperfusion
1966 Clark & Gollan	Fluorocarbon as oxygen carrier
1967 Chang <i>et al.</i>	AC with polysaccharide complexed membrane for biocompatibility
1968 Chang & Poznansky (<i>Nature</i>)	Implanted enzyme AC for enzyme therapy in inborn error of metabolism (shown in congenital catalase-deficient acatalesemic mice)
1968 Bunn & Jandl	Intramolecularly crosslinked single Hb molecule
1968 Geyer <i>et al.</i>	Fluorocarbon effective in exchange transfusion in animal studies
1969d Chang 1972a Chang	AC with lipid-polymeric membrane or lipid-crosslinked protein membrane containing cyclic transport carrier (AC contains proteins)
1970–1975 Chang <i>et al.</i>	First clinical use of artificial cells in patients (in hemoperfusion)

(Continued)

Table 1.1 (Continued)

1971a Chang (<i>Nature</i>)	Implanted enzyme AC for lymphosarcoma suppression in mice
1971b Chang	Nanobiotechnology: glutaraldehyde crosslinked Hb into PolyHb. Later, others used this method for blood substitutes in patients
1972a Chang	First monograph on <i>Artificial Cells</i>
1972b Chang (<i>Lancet</i>)	AC hemoperfusion resulted in Grade IV hepatic coma patient recovering consciousness
1973 Gregoriadis	First use of liposomes to entrap enzymes and drugs. Led to extensive development of liposomes as delivery systems
1975h Chang	Paper discussing one shot vaccine using AC
1976a Chang	Biodegradable polylactide microcapsules and microparticles containing proteins and hormones
1976 Tam, Blumenstein & Wong	Soluble dextran conjugated hemoglobin
1976 Bonhard <i>et al.</i>	Develop glutaraldehyde crosslinked PolyHb as blood substitute
1977–1985 Chang with Campbell, Cousineau, Ilan, Grunwald, Wahl, Yu etc.	Artificial cells containing multienzyme systems with co-factor recycling for multistep enzyme reactions
1978 Naito & Yokoyama	Developed perfluorodecalin as blood substitute towards clinical trials
1980 Lim & Sun (<i>Science</i>)	Alginate-polylysine-alginate AC encapsulated cells
1980 Rosenthal & Chang	AC membrane of lipid-protein-polymer containing Na^+K^+ -ATPase
1980 Djordjevich & Miller	Lipid membrane AC encapsulated hemoglobin
1985 Mitsuno, Ohyanagi	Clinical trials of perfluorodecalin as red blood cell substitute
1986 Yuan & Chang	AC containing microsomes and cytosol
1986 Bourget & Chang	Oral enzyme AC for inborn error of metabolism (phenylketonuria rat)

(Continued)

Table 1.1 (Continued)

1986 Sipehia, Bannard & Chang	AC membrane that exclude small hydrophilic molecules but permeable to large lipophilic molecules
1986 Chang, Bourget & Lister	Novel finding of extensive enterorecirculation of amino acids leading to the use of oral enzyme AC therapy to selectively remove specific unwanted systemic amino acid
1988 Tsuchida's group	Development and <i>in vivo</i> testing of synthetic heme complex either to liposome or to recombinant albumin as blood substitute
2002 Tsuchida <i>et al.</i>	
1989a Chang, 1989 Palmour <i>et al.</i> , Chang	Clinical use of oral enzyme artificial cells in a patient (patient with inborn error of metabolism: Lesch-Nyhan disease)
1989 Moss <i>et al.</i>	Clinical trials with glutaraldehyde crosslinked PolyHb
1990 Hoffmann <i>et al.</i>	Recombinant human hemoglobin
1994 Yu & Chang	Biodegradable polymeric membrane nanoartificial red blood cells
1994 Soon-Shiong <i>et al.</i>	AC encapsulated islet transplantation in a type 1 diabetic patient. Insulin independence reported
1996 Prakash & Chang (<i>Nature Med</i>)	Oral artificial cells containing genetically engineered cells lower systemic urea in a uremic rat model
1996 Aebischer, Lysaght <i>et al.</i> (<i>Nature Med</i>)	Polymeric fiber encapsulation of genetically modified xenogeneic cells for intrathecal delivery of CNTF in amyotrophic lateral sclerosis patients
1998 D'Agnillo & Chang (<i>Nature Biotech</i>)	Nanobiotechnology of crosslinking of Hb, catalase and superoxide dismutase to form soluble nanodimension PolyHb-CAT-SOD
1998 Tsuchida	Lipid AC vesicle Hb: developed and tested in animal towards clinical use
1999 Philips <i>et al.</i>	PEG-lipid membrane AC containing Hb increases circulation time
2000 Liu & Chang	AC coencapsulating hepatocytes and adult stem cells
2001 Lörh <i>et al.</i> (<i>Lancet</i>)	Clinical trial of AC microencapsulated cell-mediated treatment of inoperable pancreatic carcinoma in patients
2002 Gould <i>et al.</i>	The life-sustaining capacity of human polyhemoglobin in trauma surgery clinical trials
2002 Sprung <i>et al.</i>	The use of bovine polyhemoglobin in surgical patients: results of a multi-center, randomized, single-blinded trial

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Table 1.1 (Continued)

2003 Chang, Powanda, Yu	PEG-PLA membrane nanodimension AC containing Hb and rbc enzymes
2004 Bloch <i>et al.</i> , Aebischer	Phase I clinical study for Huntington's Disease, using encapsulated cells engineered to secrete human ciliary neurotrophic factor
2004 Yu & Chang (<i>Melanoma Res J</i>)	Nanobiotechnological approach of PolyHb-tyrosinase: delays the growth of melanoma in a rat model
2006 Liu & Chang (<i>J Liver Trans</i>)	AC encapsulated bone marrow stem cells regenerate liver resulting in recovery and survival of rats with 90% of liver surgically removed

(Updated from Chang 2005, *Nature Review Drug Discovery*).