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Research Article

ARTIFICIAL CELL

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The **artificial cell** or **minimal cell** is an engineered particle that mimics one or many functions of a biological cell. It is an idea that certain functions or structures of biological cells can be replaced or supplemented with a synthetic entity. Artificial cells are biological or polymeric membranes which enclose biologically active materials. Notably, the artificial cells have been clinically successful in hemoperfusion, implantation, blood transfusion and many other clinical conditions. In the area of synthetic biology, a "living" artificial cell has been defined as a completely synthetically made cell that can capture energy, maintain ion gradients, contain macromolecules as well as store information and have the ability to mutate. Such a cell is not technically feasible yet, but a variation of an artificial cell has been created in which a completely synthetic genome was introduced to genomically emptied host cells. Although not completely artificial because the cytoplasmic components as well as the membrane from the host cell are kept, the engineered cell is under control of a man-made genome and is able to replicate.

INTRODUCTION

A cell is considered as fundamental unit of all living organism. A single cell costitutes an entity and separated from other neiboring ons by a cell membrane. It also contains a variable sub-cellular structures and chemical material, which make it able to function. Two fundamental types of cells are eucaryotes, which are cells that contain a nucleus like most animal and plant cells, and procaryotes (bacteria), which are cells having no nucleus (Figure 1) . [1].

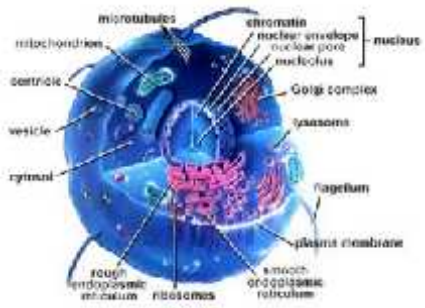


Fig. 1 Schematic structure of an animal cell [Wong and Chang,1988].

Artificial cell is not a specific physical unit only but it is considered as an idea concerning its preparation from artificial structures. These structures must have cellular dimensions for possible replacement or supplement of the deficient cell functions. Artificial cells could be microscopic aggregations of simple organic and inorganic molecules that assemble and replicate themselves autonomously [2].

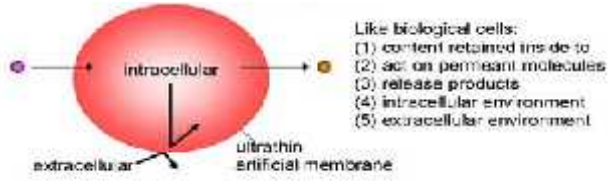
Artificial cells are basic cells that have been created artificially and enhanced with packages to be used by the body. They constructed from water-insoluble man-made particles that can achieve specific bio-functions in the body without being rejected by the defense system. They can be used to treat disease by introducing small medical devices [3].

Basic features of early artificial cells

The former artificial cells have some simple properties of the biological cells:

The membrane, that separates its internal environment from the outside, can be prepared to selectively allow only different types of molecules to cross it.

Basic principles of early artificial cells



The cell membranes of artificial cells are thin, strong and have a large surface area like the total membrane surface area of an artificial kidney machine. Additionally, this membrane is 100 times more thinner than the one of an artificial kidney

membrane. This means that any small molecules can move across 10 ml of 20 *μm* diameter artificial cells 100 times faster than that move across an artificial kidney machine.

Artificial cells constitute from the same biological material as the biological cells. As well as, they are more versatile so a any dsorbents, magnetic materials, drugs and other material can also be incorporated either separate or in combination [6,10].

Historical background

1950s

Professor MacIntosh, chairman and Sir Arnold Burgen had started a special "honors physiology" program in the faculty of medicine at McGill University. This challenging program had combined the progress of cell physiology with the advance courses in polymer chemistry, physical chemistry and radiation chemistry. It began the interest in applying basic research to the medical treatment. [11,12].

1960

The first artificial cells were established by Thomas Chang at McGill University . They were micron-sized and consisted of ultrathin membranes of nylon, collodion or crosslinked protein that had semipermeable properties These cells contained cell, enzymes, hemoglobin, magnetic materials, adsorbents and proteins [13]

Later on, the artificial cells size have ranged from hundred-micrometer to nanometer dimensions and could be loaded by microorganisms, vaccines, genes, drugs, hormones and peptides. The first trial of clinical use of artificial cells was in hemoperfusion by the encapsulation of activated charcoal [14].

1970s

Researchers could also introduced enzymes, proteins and hormones to biodegradable microcapsules. This way helped them clinically in some diseases such as Lesch-Nyhan syndrome [15].

1990s

Artificial and biodegradable artificial red blood cells were also developed.

1994

In diabetic patients, the artificial cells in biological cell encapsulation were first used in the clinic for treatment. Furthermore, hepatocytes, adult stem cells and genetically engineered cells have been encapsulated and were under the study and trials for the use in tissue regeneration [16,17]

2011

At Harvard University, chemists had reported the creation of an artificial cell membrane [18]

2014

Researchers could produce self-replicating, synthetic bacterial cells with cell walls and synthetic DNA. In January of that year, They produced finally an artificial eukaryotic cell capable of undertaking multiple chemical reactions through working organelles [19,20]

Aim

Synthetic biology (SB) is a recently emerging cell biology branchwhich combines areas of engineering, chemistry, biotechnology, computer science and molecular biology. It aimed programmable construction of biological parts, devices and systems to perform useful functions.

The goal of synthetic biology is building a "living" artificial cell and open up new fields of possible applications of it in medicine. A science should improve the lives of people with new, low-cost and effective methods of environmental protection and treatment of diseases.

This article aimed to highlight the main scientific issues influenced by the public controversies of social and ethical landscape about genetic engineering and nanotechnology of artificial cells applications.

Focusing on the intrinsic features of artificial cells creates a suggestion that constructing artificial cells is unnatural. It commoditizes life, that it promotes a reductionist standpoint, that it is performing the role of God, and that we should use religious texts as sources of authority**.**

Preparation

Many variable methods for artificial cell preparation and encapsulation have been developed. Typically, vesicles such as a nanoparticle, polymersome or liposome were manufactured. An emulsion is typically made through the use of high pressure equipment such as a high pressure homogenizer or a Microfluidizer. There are two micro-encapsulation methods for nitrocellulose that will also described below [21,22].

High-pressure homogenization

In a high-pressure homogenizer, there are two liquids in oil/liquid suspension forced through a small orifice under a very high pressure. This process shears the products and so allows the creation of the extremely fine particles, as small as 1 nm.

Microfluidization

This technique uses a patented Microfluidizer to get a greater amount of homogenous suspensions. It also can create smaller particles than homogenizers. First, homogenizer is used to create a coarse suspension that is then pumped into the microfluidizer underneath high pressure. Then, the flow is split into two streams that will react at very high velocities in an interaction chamber until desired size of the particle is obtained. By this technique, large scale production of phospholipid liposomes and subsequent material nanoencapsulations could be obtained.

Drop method

In this method, a cell solution is incorporated dropwise into collodion solution of the cellulose nitrate. As the drop moves through the collodion, it is coated with a membrane due to the interfacial polymerization properties of the collodion. Later, the cell settles into paraffin where the membrane arrays and is finally suspended a saline solution. The drop method is intended for the creation of large artificial cells that encapsulate the biological cells, stem cells and genetically engineered stem cells.

Emulsion method

This method differs because the material used to be encapsulated is smaller and is putted in the bottom of the reaction chamber. In this chamber, the collodion is added on top and centrifuged, or otherwise disturbed in order to generate an emulsion. The encapsulated material is then dispersed and suspended in the saline solution**.**

Characteristics of artificial cells

The fundamental characteristics of artificial cells, namely cell body (material and structure), its biofunctionality, and the bioinert surface with its homing device.

Artificial cell body

The architecture of the artificial cell body can be nano- or micro-particles including micelles, capsules, liposomes and polymersomes. This allows it to include a large amount of biofunctional material and/or to prevent degradation, solubility or stability problems of the biofunctional material. For applications in the circulation, the size of artificial cell should be smaller than 1-2 μm to prevent obstruction of the capillaries. On the other hand, non-biodegradable polymers remain in the body after the cells have performed their function, thus a specific treatment may be required to remove these cells. Therefore, biodegradable polymers such as polylactides (PLA), poly(lactic-co-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL), which do not show major adverse reactions (depending on the application) should preferably be used as the cell body material[23,24]. (Fig.7)

Biofunctionality

The bio-function of the artificial cells means that their bio-functional material including in these cell can locally alter their physiological events and/or a physical parameter. *For example*,

- Preventing tumor cells to grow made by delivering anticancer drugs
- Treating enzyme defects in inborn problems of metabolism by delivering encapsulated enzymes
- Modulating the expression of a gene done by delivering oligonucleotides,
- Transporting O_2/CO_2 or changing the electron density so enhancing the contrast of certain organs.

An artificial cell body can be provided with biofunctionality by encapsulation or immobilization of many different kinds of biofunctional materials as drugs, enzymes, peptides or DNA. The encapsulation of genetically engineered cells by a semipermeable membrane may avoid the adverse immune response in non-autologous cell therapy. Cell encapsulation has supplied a range of promising curative treatments for some diseases such as diabetes, hemophilia, cancer and renal failure. The perfect approach could be by encapsulating the cells and implant them into the body for long-term functioning. Microencapsulated islets have been implanted in diabetic animals and humans to maintain the normal glucose levels. Also, implanted microencapsulated hepatocytes can decrease the elevated bilirubin level in Gunn rats with an inborn error of bilirubin metabolism [32].

Bioinert surfaces

The main problem that is encountered with the application of artificial cells in the circulation is the rapid clearance by the MPS including the adhesion to or phagocytosis by the endothelial lining of the vascular system. In order to prevent rapid clearance and thus to provide a prolonged circulation time, artificial cells should have favorable surface properties that upon introduction of the cells in the circulation induces as little protein (especially opsonins) adsorption and complement activation as possible. Protein adsorption and complement activation can be reduced by grafting the cell surface with hydrophilic, nonionic polymers, such as natural materials, e.g., proteins, polysaccharides and synthetic polymers, e.g., poly (hydroxyethyl methacrylate) and PEG. [33,34].

Site specific targeting

A homing device on an artificial cell surface be able to give the cell specificity by guiding the cell to the site where it is intended to perform its function. The drug targeting principal schemes include direct drug administration to the pathological site and/or passive drug accumulation via leaky vasculature (tumors, inflammation). The physical targeting of the drug is based on the abnormal pH value and/or temperature in the pathological zone, magnetic targeting, and targeting using specific ligands which have an increased affinity for this area of interest. Microcapsules, microparticles, liposomes, and micelles are pharmaceutical carriers and have been successfully used for targeted drug delivery, making use of proteins including antibodies (AB) and AB fragments, lipoproteins, lectins, hormones, and polysaccharides as targeting moieties. The most effective ways of targeting antigen expressing cells and subsequent specific uptake of these particles by the target cells are performed by providing a particle surface with a monoclonal antibodies. This approach can be generally applied to improve the therapeutic efficacy of anticancer drugs by reducing the drug side effects and to design advanced diagnostic systems [35,36].

Nanobiotechnology and artificial cells

So, constructing an artificial cell that organizes and sustains itself and evolves to its environment would open the door to generating several technologies with the impressive capabilities of living systems for social and economic gain [37]**.**One of these technologies is nanobiotechnology.

Nanotechnology is the science of nanoscale structures. While, biotechnology can deal with metabolic process of micro-organisms. Merging of these two technologies results in evolution of nano-biotechnology. It means assembling of biological molecules into nanodimension structures, nanodimension thickness of membranes or nanotubules with nanodimension diameter. This biotechnology is used to form the membrane of artificial red blood cells [38].

The usefulness of nanotechnology to biomedical sciences indicate creation of materials and devices that were designed for interacting with the body at the sub-cellular scales and with a high degree of specificity. This could be potentially interpreted into targeted cellular and tissue-specific clinical applications that aimed at maximal therapeutic and curative effects with very restricted adverse-effects.

One of most promising applications of nanotechnology is in the perspective of medicine. Indeed, a whole new field of

"nanomedicine" is emerging. Nanomedicine has been defined as the monitoring, repair, construction and control of the human biological systems at their molecular level by using engineered nanodevices and nanostructures [39].

Most of the original ideas and basic research were interrelated to enzyme and gene therapy, cell therapy, blood substitutes, regenerative medicine and nanomedicine. All these developing techniques were valuable for actual clinical use and required parallel developments in the molecular biology and biotechnology that were not yet available. More newly, many groups around the world have complete exciting progress in biotechnology, molecular biology, genetic engineering and related areas. The outcome of this progress is a recent new era of research and the constructing the artificial cells[40].

Clinical application of artificial cell

Artificial Red Blood Cells (RBCs): (Fig. 11)

Oxygen carriers (Haemoglobin-based oxygen carriers)

Oxygen carriers of nano-sized are used as a type of red blood cell substitutes but they lack the other components of red blood cells. 44,45]

Red blood cells

Attempts have been made to develop a complete working red blood cell that includes an oxygen carrier, carbonic carrier and also the enzymes associated with the cell. In 1957, it was the first attempt to make RBCs by replacing the membrane of red blood cell by an ultrathin polymeric membrane. This trial was followed by cell encapsulation through a lipid membrane [53] and more recently a biodegradable polymeric membrane.[47] An biological red blood cell membrane containing lipids and its associated proteins can also be used to encapsulate the nanoparticles and increase the residence time in vivo. This made by bypassing the uptake by macrophage and the systemic clearance.[48] [49]

separated from the rest of the cells in a centrifuge. Their next step will be to trial the blood in patients in 2016.

Enzyme therapy

Enzyme therapy is being vigorously studied for genetic metabolic diseases. In these diseases, an enzyme is either over-expressed, under-expressed, defective, or not at all there. In the condition of under-expression or expression of a defective enzyme, an active form of this enzyme is introduced in the body to compensate for the present deficit. On the other hand, an enzymatic over-expression may be counteracted by introducing a competing non-functional enzyme which metabolizes the substrate into non-active products. When the enzyme placed within an artificial cell, it can carry out its function for a much longer period as compared to the free one and also can be further optimized by polymer conjugation.[50].[51.52]

Encapsulation of artificial cells with the cytochrome p450 enzyme that converts the prodrug into the active drug can be designed to accumulate in the pancreatic carcinoma or implanting the artificial cells close to the tumor site. Here, the local concentration of the activated enzyme will be much higher than in the rest of the body so preventing any systemic toxicity. This treatment was successful in both man and animals and displayed a doubling in median survivals among the advanced-stage pancreatic cancer patients who in phase I/II clinical trials, and a tripling in one-year survival rate.[51,52]

Gene therapy

This therapy aims to insert, alter or remove genes within an afflicted individual's cells. [53-54].

Artificial cells have been planned as a non-viral vector. Through this vector, the genetically modified non-autologous cells are encapsulated and implanted to deliver recombinant proteins *in vivo* [55].

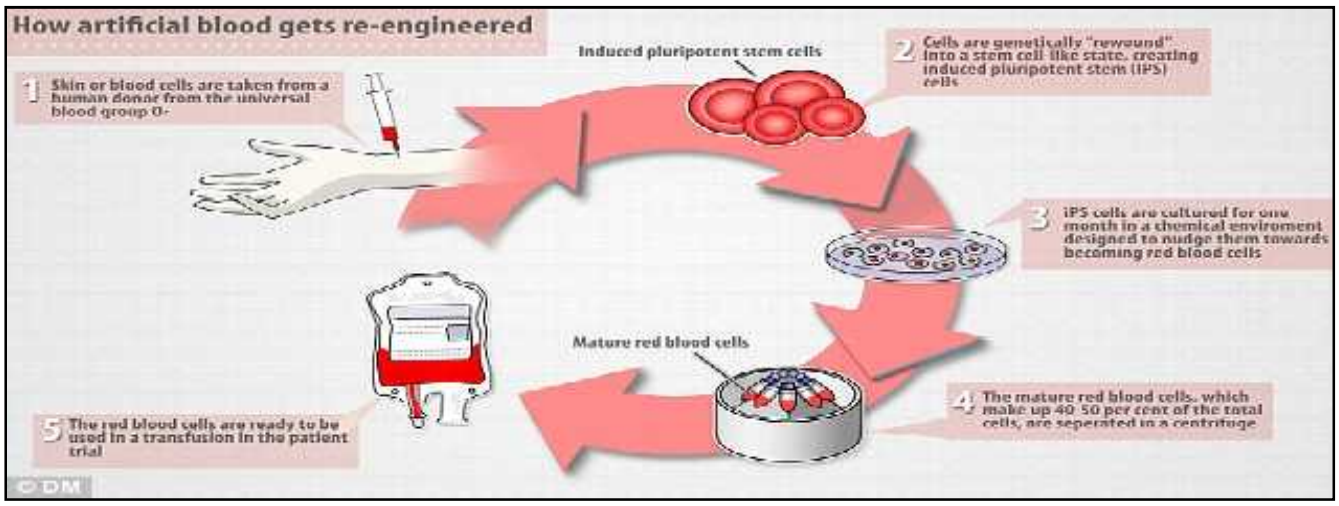


Fig. (11): The process involves using adult skin or blood cells that have been genetically modified into stem cells, known as induced pluripotent stem (iPS) cells. These iPS cells are then cultured in biologic conditions that mimic the human body, eventually leading to their transition into mature red blood cells. The trick so far has been increasing the efficiency of this transition process, as not all the cells are capable of becoming red blood cells. The red blood cells are then

This type of immuno-isolation is efficient in mice through delivery of artificial cells that contain mouse growth hormone intended for rescued a growth-retardation in mutant mice. A few strategies have been advanced to human clinical trials for the treatment of pancreatic cancer, lateral sclerosis and pain control [56].

Hemoperfusion

The first clinical use of artificial cells was in hemoperfusion through the encapsulation of activated charcoal [13].

Artificial cell hemoperfusion has been offered as a less costly and more efficient detoxifying option than hemodialysis. In this way the blood filtering takes place only through size separation by a physical membrane. In hemoperfusion, there are thousands of adsorbent artificial cells could be retained inside a small container. This performed through the use of two screens on either end through which the patient blood peruses. This device has been established as a routine clinical method for those patients treated for accidental or suicidal poisoning as well as introduced as therapy in the liver and kidney failure by carrying out part of their function [57]. Hemoperfusion is considered useful especially in the countries with a weak hemodialysis manufacturing industry because these devices tend to be cheaper there and used in kidney failure patients.

Artificial cells for cell encapsulation

By the use of membrane systems, different attempts have been made to prevent the rejection of transplanted cells by the immune system. These trials include the use of dialysers, ultrafiltrators, membrane sacs, membrane disks and polymeric devices. However, in these patterns, the low viability of cells due to low oxygen and nutrients permeability is one of the major problems. In 1964, Chang successfully used bioencapsulate cells to protect the enclosed cells from immunorejection[12].

Artificial cells containing hepatocytes and/or stem cells in regenerative medicine

The shortage of organ donors sort the artificial cells as key players in alternative therapies for liver failure. The use of artificial cells for hepatocyte transplantation has demonstrated feasibility and efficacy in providing the normal liver function in animal models with liver disease and bioartificial liver devices.[58][66][67]. [68],[69] 70] [71] 72] 73] [74] [75]

Potential risks and benefits

Additionally, many technological, economic, and social benefits grouped to the scientific benefits of AC because they would be a threshold technology that opens the door to new kinds of applications. Also, artificial cells may designed for specific applications that offer unprecedented opportunities for biotechnology. They allow us to combine the properties of biological systems such as nanoscale efficiency, self-organization with the adaptability for therapeutic and diagnostic applications. So, it will become promising to construct communities of these AC that can self-organized to achieve many different tasks and even evolved in response to any changes in the environment[59,60].

Artificial cells also increase the significant social and ethical worries. There are a long history of ethical issues related to creation of artificial forms of life, dating back at least to the artificial production of urea which is the first man-made organic compound. Fears about nanostructures proliferating in natural environments were expressed in the nanotechnology community a decade ago [61]**.** A recent cautionary piece by Bill Joy in *Wired* about the combination of nanotechnology along with genetic engineering [62] flashed extensive commentary on the web.

The artificial cells potential to threat the human health and the environment is one of the most wide-spread worries. Also, there was another fear about the molecular machines that had the ability to reproduce themselves and evolve uncontrollably. Referring to the important dangers of the genetic engineering with the **Eric Drexler's** [63] warnings about the dangers of self-reproducing nanotechnology, **Joy** [64] concluded that "this is the first moment in the history of our planet when any species, by its own voluntary actions, had become a danger to itself—as well as to vast numbers of others,"

As Drexler explained: "Plants" with "leaves" no more efficient than today's solar cells could out-compete real plants, crowding the biosphere with an inedible foliage. Tough omnivorous "bacteria" could out-compete real bacteria: They could spread like blowing pollen, replicate swiftly, and reduce the biosphere to dust in a matter of days. Easily, dangerous replicators could be too tough, small, and rapidly spreading to stop-at least if we make no preparation. We have already trouble enough controlling viruses and fruit flies.

Joy adds the threat of new and vastly more lethal forms of bioterrorism to the health and environmental risks of artificial cells

These dangers impersonated by AC stem from two key features. First, since they would be self-replicating so if they pose any danger, there is a potential to be magnified on a vast scale as the artificial cells proliferate and spread around the globe. Second, because they would be evolving, their properties could be changed in the ways which we never anticipated. For example, they could evolve new ways of competing with existing life forms and new ways to evade our eradication methods. This potential for an open-ended evolution makes the long-term consequences of creating themselve extremely unpredictable. Much of the positive potential of artificial cells stems from their capacity to replicate and evolve and the very same power raises the specter of life run amok.

CONCLUSIONS

Artificial cells are in our future. That future could come within this or another decade. By coupling the automatic regeneration and spontaneous adaptation of life, the artificial cells promise our society a wide variety of social and economic benefits. But, their ability to self-replicate and unpredictably evolve create unpredictable risks to human health and the environment. So it behooves us to start

Future prospects of artificial cell

Electronic artificial cell" Programmable Artificial Cell Evolution" (PACE) program from **2004-2008 ,**whose goal was to lay the foundation for the creation of "microscopic self-organizing, self-replicating, and evolvable autonomous entities built from simple organic and inorganic substances that can be genetically programmed to perform specific functions" [59] Following this research, in 2007, John McCaskill proposed to concentrate on an electronically complemented artificial cell, called the **Electronic Chemical Cell**. The key idea was to use a massively parallel array of electrodes coupled to locally dedicated electronic circuitry, in a two-dimensional thin film, to complement emerging chemical cellular functionality.

Recombinant technology will continue to generate valuable nonpathogenic genetically engineered bacterial cells and other genetically engineered cells that have potentially therapeutic functions or that produce therapeutic products useful in the treatment of disease.

Artificial blood that could one day be used in humans without side-effects was created by scientists in Romania towards the end of last year. The blood contained water and salts along with a protein known as hemerythrin that is extracted from sea worms.

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